Antioxidant, Antimicrobial and Antiscalant Properties of Selected Species of *Ficus*

By

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DEDICATION

This Endeavors Is

Dedicated To

My

Beloved Family

for their Sacrifices, Support

and Continuous Prayers

ACKNOWLEDGEMENTS

I bow my head before **Almighty Allah**, The omnipotent, The most gracious, The compassionate, The beneficent, who is the entire and only source of every knowledge and wisdom endowed to mankind and who blessed me with the ability to do this work. It is the blessing of **Almighty Allah** and **His Prophet Hazrat Muhammad** (Peace be Upon Him) that enabled me to achieve this goal.

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ABSTRACT

World over, *Ficus* is recognized to be an important genus containing more than 850 species, out of which 14 are native to Pakistan. *Ficus* species are widely distributed and easily available throughout Pakistan. Different parts of the *Ficus* plants have been used in folk medicines since centuries. These are reported to possess multiple such as

antidiarrheal, antidysenteric, antidiabetic antiasthmatic, antimicrobial, analgesic, laxative, and haemostatic activities. The present work has been designed with the main purpose to evaluate antioxidant, antimicrobial and antiscalant properties of different parts of selected species of *Ficus* distributed in Pakistan. Four solvents (absolute ethanol, absolute methanol, 80% ethanol, 80% methanol) and three techniques (orbital shaker, magnetic stirring, and sonication assisted extraction) were employed to evaluate the influence of extraction process on the yield and activities of extractable components. Antioxidant properties of the extracts were evaluated following different *in-vitro* antioxidant assays such as determination of total phenolic contents, estimation of total flavonoids, measurement of DPPH radical scavenging capacity, measurement of %age inhibition of linoleic acid peroxidation and reducing potential. The most potent extract from each sample was used to identify and quantify individual phenolic acids and flavonoids with the help of RP-HPLC. Furthermore, the most potent extract for each sample was also evaluated for its antimicrobial and antiscalant activities. Antimicrobial activities were assessed against selected strains of bacteria and moulds using disc diffusion and resazurine indicator methods. Antiscalant activity of the extracts was evaluated through conductivity measurement and by microscopic examination. Sonication assisted extraction technique and 80% methanol proved to be the most effective extraction system and offered higher extraction yields of active components as compared to other combinations of technique and solvent employed. The extracts obtained by using 80% methanol as solvent and sonication as extraction technique also constituted higher total phenolic and total flavonoid contents as well as exhibited better antioxidant activities as compared to the extracts obtained by other combinations of solvent and extraction techniques. HPLC analysis revealed the occurrence of four phenolic acids (caffeic acid, chlorogenic acid, gentisic acid and sinapic acid) and two flavonoids (rutin and quercetin) in almost all the fruit, leave and bark samples of the *Ficus* species investigated. Leaves and fruit extracts of the selected samples exhibited moderate antibacterial activity although none of the sample demonstrated antifungal activity. All the samples showed fairly good antiscalant activity. Overall, from the findings of this comprehensive study, it could be understandable that an appropriate extraction system is necessary for recovery of optimum amount of potent antioxidants, and antimicrobial and antifungal agents from these materials. The present results advocate the utilization of different parts of the local *Ficus* species for isolation of valuable bio-actives for functional food, neutralceutical and water treatment plants.

Chapter-1

INTRODUCTION

1.1. Lipid Oxidation

Among the compounds of organic nature, lipids are most prone to oxidation. Lipid oxidation is a severe problem both for living systems and the food industry. It is one of the major causes leading to generating reactive oxygen species (ROS) which are linked with several health disorders and food deterioration (Ceruitti, 1991; Yıldırım *et al.,* 2001; Wang *et al.,* 2002; Jeong *et al.,* 2004; Sultana *et al.,* 2007). Food quality is deteriorated by the process of oxidation during processing and storage. Reactive oxygen species, generated during this process, react with nutrients in food producing stable oxidation products which are dangerous to health being carcinogens (Oliveira *et al.,* 2009). Due to these oxidative products nutritional quality and organoleptic value of foods is decreased making the food unacceptable to consumers.

Moreover, lipid-soluble vitamins and essential fatty acids can be degraded by lipid oxidation products. Proteins and amino acids can react with secondary oxidation products like malondialdehyde and 4-dihydroxynonenal and as a result undesirable colour darkening and textural changes can occur in food (Kanner and Rosenthal, 1992).

The process of lipid oxidation in food and other systems completes in three stages which are initiation, propagation and termination. Free radicals are initiated in first stage under the influence of light, singlet oxygen, transition metals and already existing free radicals. The formation of free radicals is accelerated in the propagation stage, free radicals generated in first stage react with nutrient molecules to produce hydroperoxide molecules (ROOH) and also generate another free radical (R^*). In termination step free radicals react with each other and generate more stable non radical molecules and in this way complete one cycle of oxidation. However cycle can be repeated due to re-initiation (Kanner and Rosenthal, 1992). Hydroperoxide molecules (ROOH) are also called primary oxidation products and these are highly unstable molecules. Due to their instability, Hyderoperoxide molecules are further degraded and generate ketones, aldehydes, alcohols and carboxylic acids which are also called secondary oxidation products. These secondary oxidation products are responsible for the development of off-flavor and offodor in oxidized foods.

1.2. Antioxidants

It is an established fact that in biological systems under oxidative stress, ROS can damage bio-molecules (Valko et al., 2007) and this may increase the risk of more than 100 diseases (Perry *et al.,* 2000; Yıldırım *et al.,* 2000). Antioxidants are used to mitigate the damages caused by ROS in foods and in biological systems.

"Antioxidants are highly oxidizable substances which can delay or inhibit the process of oxidation in a substrate even at low concentration (Halliwell and Gutteridge, 2007)." Antioxidants can use different mechanisms to fight against oxidative stress. On the basis of mechanism they adopt, antioxidants are divided in to primary antioxidant and secondary antioxidant. Primary antioxidants are also known as chain breaking antioxidants as shown in figure 1.1. They can trap the free radical in initiation or propagation steps of oxidative sequence and thus prevent or slow down the process of oxidation.

$$R^{\bullet} + AH \longrightarrow RH + A^{\bullet}$$

$$RO^{\bullet} + AH \longrightarrow ROH + A^{\bullet}$$

$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$

$$ROO^{\bullet} + A^{\bullet} \longrightarrow ROOA$$

$$RO^{\bullet} + A^{\bullet} \longrightarrow ROA$$

$$A^{\bullet} + A^{\bullet} \longrightarrow AA$$

Figure 1.1. Mechanism of reaction of primary antioxidant

Secondary antioxidants are also termed as preventive antioxidants. They exhibit their activity to slow down the oxidation process through various mechanisms e.g. they can act as metal chelators, absorbs UV radiations, singlet oxygen quencher or can scavenge oxygen like metal chelation.

Antioxidants are applied in food during processing for the prevention of offflavors, rancidity, and similar phenomena and to increase the shelf life of the food. For this purpose many efficient and cost effective synthetic antioxidants e.g. propyl gallate (PG), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and butylated hydroxytoluene (BHT) were developed. However, different researches on synthetic antioxidants showed that they might be implicated in many risks related to health including cancer (Jeong *et al.,* 2004; Iqbal, Bhanger and Anwer, 2007). Hence, many studies have been prompted to substitute the synthetic antioxidants with components having antioxidant attributes from natural sources and which do not have any risk (Paradiso *et al.*, 2008; Descalzo and Sancho 2008).

1.2.1. Plants: A viable source of natural antioxidant

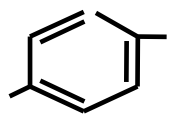
Plants are the richest source of natural antioxidant (Shahidi, 1997). Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals (OH^-) or superoxide radical (O^{-2}) scavengers (Govindarajan *et al.*, 2005; Robards *et al.,* 1999). Some antioxidants are also strong chelators of metal ions (Evans *et al.,* 1995). Thus, a practical way to control these diseases is to increase the dietary intake of plant based foods which are rich sources of antioxidants (Demo *et al.,* 1998; Proteggente *et al.,* 2002; Sun *et al.,* 2002).

1.3. Phenolics

It is an established fact that phenolic contents present in plant extracts are mainly responsible for the antioxidant activity of the plant extracts (Heim *et al.,* 2002). Phenolic compounds are biologically active plant secondary metabolites (Randhir and Shetty 2004). Different types of physiological and therapeutic properties have also been shown by phenolic compounds in human, such as antioxidant, antiartherogenic, anti-allergenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects (Devasagayam *et al.*, 2004; and Manach *et al.*, 2005).

Phenolic compounds are widely distributed in plant kingdom, however, their concentration vary from plant to plant or even in different organs of the same plant. Many plants are considered as excellent sources of phenolic compounds that could be used, not only to preserve foods, but also to contribute to a healthy diet (Liu *et al.*, 2008; Tabata *et al.*, 2008). Among various phytochemicals, dietary phenolic compounds such as ferulic, *p*-coumaric, sinapic and syringic acids and polyphenols typically, flavonoids (flavonols, flavones etc) are considered to be powerful antioxidants. Their antioxidant activity is much higher in vitro than of well-known vitamin antioxidants (Palanisamy *et al.*, 2008).

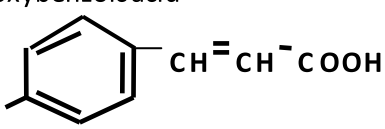
Molecular structures of some important phenolic acids and flavonoids are shown in figure 1.2 and 1.3 respectively. Flavonoids constitute approximately two-third of the total dietary phenolics. Among different classes of flavonoids, flavonols i.e. quercetin, myricetin, and kaempferol are most important due to their important physiological functions and health benefits (Benbrook 2005).



H

(a) *P*-Hydroxybenzoic acid

(d) *P*-Coumaric acid

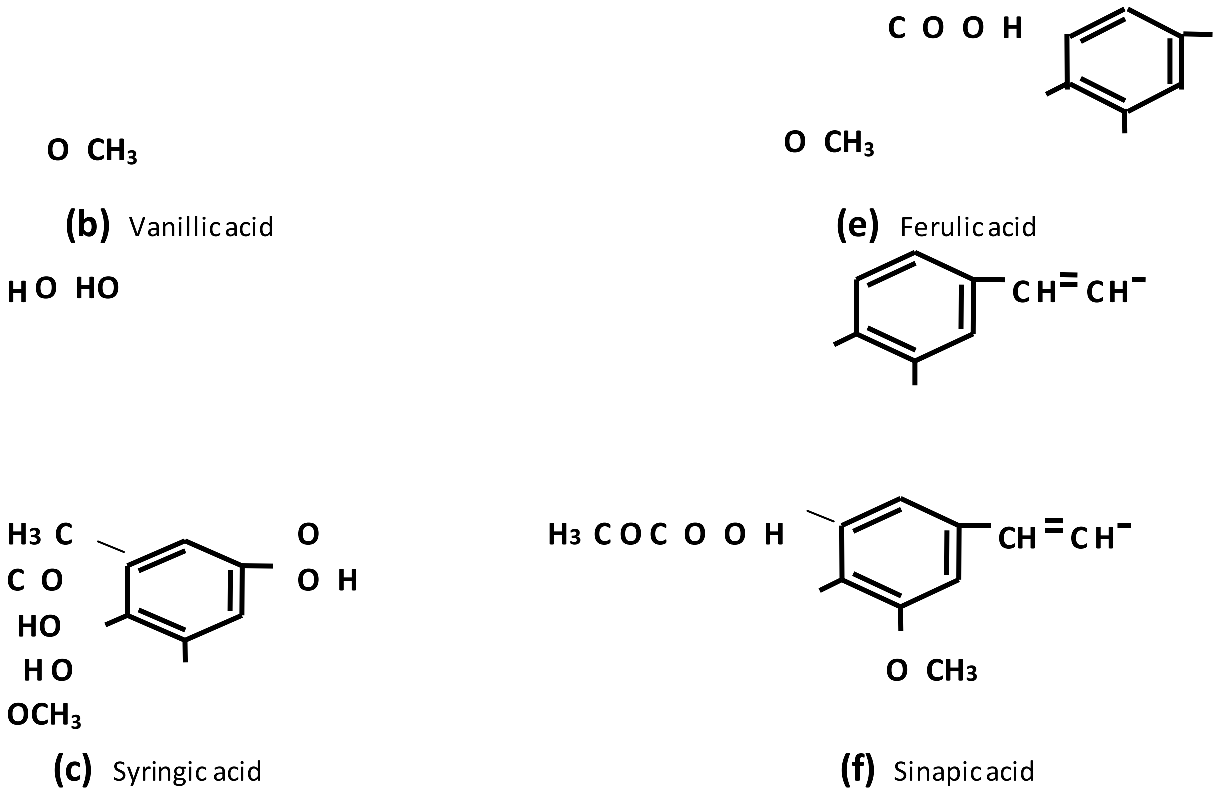


COOH

O

HO

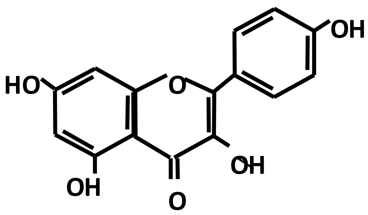
COOH



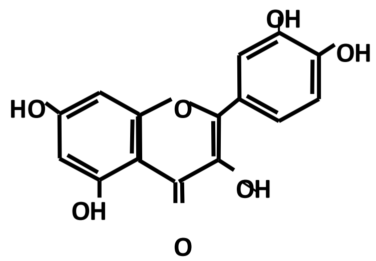
(a) to (c) Benzoic acid derivatives

(d) to (f) Cinnamic acid derivatives

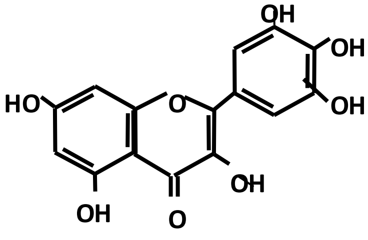
Figure 1.2. Structure of some important phenolic acids (Hakkinen 2000)



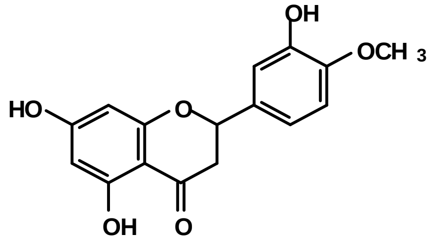
Kaempferol



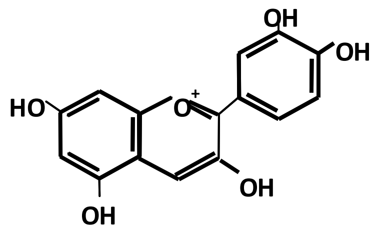
Quercetin



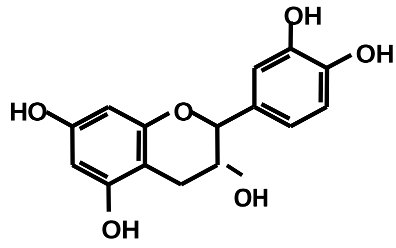
Myricetin



Hesperetin



Cyanidin



Epicatechin

Figure 1.3. Structure of some important flavonoids (Hakkinen, 2000)

1.4. Antimicrobials

Plants, animal and human suffered from different kind of infectious diseases because of microorganisms. Contamination of food with food born microbes is also a serious concern to human health and diseases caused due to these contaminations is a major problem even in the developed countries (Sokmen *et al*, 2003). Quality of food can be deteriorated by the presence of a variety of microbes which is a major concern to the food industry (Sokmen *et al,* 2004). Raw and/or processed foods are open to contamination during the production, sale and distribution of the foods (Deak and Beuchat, 1996). Thus, at present, it is a necessity to use the chemical preservatives to prevent the growth of food spoiling microbes in the food industry (Ozcan, 2003).

Antibiotics and synthetic drugs are effectively used for curing of many infectious diseases (Barbour *et al,* 2004). Antibiotics are secondary metabolites produced by certain groups of microorganisms and their effect may be cidal (can kill the microbes) or static (can inhibit the growth of microbes) on a range of microorganism (Burt, 2004).

At present the pharmaceutical drugs available to control antibiotic-resistant bacteria are becoming limited. The indiscriminate use and abuse of antibiotic has led to the development of antimicrobial resistant strains and some of these drugs are also toxic to human and animals (Essawi and Srour, 2000; Parekh and Chanda, 2007). Drug resistance of human and animal pathogenic microbes and parasites has created a serious problem worldwide as previously treatable ailments such as tuberculosis and diarrhea are now more difficult and expensive (Dwyer et al, 2009).

Due to the economic impacts of spoiled foods and the consumer's concerns over the safety of foods containing synthetic chemicals, a lot of attention has been paid to naturally derived compounds or natural products (Alzoreky & Nakahara, 2003; Hsieh, Mau, & Huang, 2001). Plants are rich in a wide variety of secondary metabolites such as flavonoids, tannines, many of which have been found to exhibit in vitro antimicrobial activities (Cowan, 1999; Lewis and Ausubel, 2006). Medicinal plants belonging to different parts of world e.g. Kenya also have antimicrobial activities (Matu and Van Staden, 2003; Bii *et al*, 2008; Kareru *et al*, 2008). Medicinal plants have also featured as therapeutic agents used by the world population for basic health care needs and to combat many kind of infectous disease worldwide (Voravuthikunchai and Limsuwan, 2006). Due to the pathogens resistance against the available antibiotics and the recognition of traditional medicine as an alternative form of health care has reopened the research domain for the biological activities of medicinal plants (Arias *et al.*, 2004).

1.5. Scaling and Antiscalants

Deposition of mineral scales (e.g., $CaCO_3$, $CaSO_4$, and $BaSO_4$) is a serious practical problem in many process industries (Neville *et al.*, 1999). Deposition of $CaCO_3$, poses particular problems on heated surfaces in boilers, cooling water systems and desalination plants (Neville *et al.*, 1999) such as reduction in the permeate flux (Abd-ElAleem *et al.*, 1998) decrease in plant life, increase in energy consumption and high cleaning frequency (Li *et al.*, 2006).

One of the most common methods used to prevent or control the scaling of different salts in water feed is the addition of scale inhibitors also known as antiscalants. Antiscalants: they are surface active materials that interfere with precipitation reactions in three primary ways:

One of these processes is threshold inhibition; It is the ability of an antisclant to keep supersaturated solutions of sparingly soluble salts. The second method through which antiscalants inhibit the scaling is crystal modification, through this mechanism, the antiscalant molecules used their negative sites to attack the positive groups on scaling nuclei when they start to grow at sub microscopic level and in this way distort the electronic balance which is essential for the growth of crystal. In this way geometry of crystal is interrupted due to which soft and non-adherent

scale is produced. Scale crystals grow more oval in shape and less compact when treated with some crystal modifiers. This mechanism is graphically represented in figure 1.4. In third mechanism which is called dispersion (shown in figure 1.5), antiscalant molecules are adsorbed on the colloidal particles on the surface of crystals and in this way an anionic charge is imparted. Due to this high anionic charge crystals remain separated not only from each other but also from the membrane surface due to presence of fixed anionic charges on it (Abdel-Gaber *et al.*
2008).

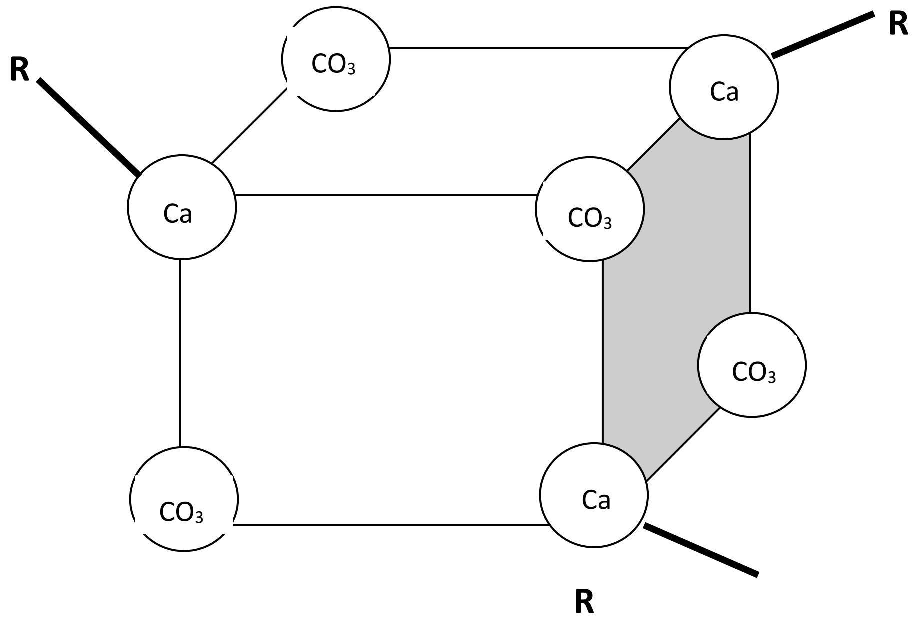


Figure1.4. Threshold mechanism (www.lenntech.com/antiscalants.htm)

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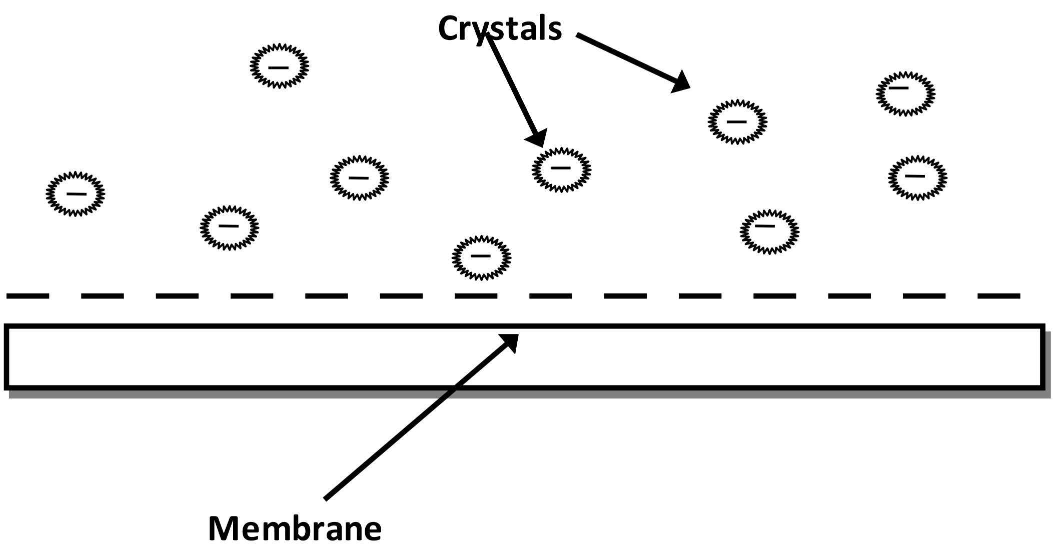


Figure 1.5. Dispersancy (www.lenntech.com/antiscalants.htm)

Antiscalants have been widely applied for many years in reverse osmosis membranes, heat exchangers, water cooling and desalination plants to prevent scaling and improve plant performance and have proven to be an efficient measure. At present, there are various antiscalants available in the market, but most of them have problems such as high cost and serious environmental influence, which have been the focus of attention. Currently plant extracts are also gaining importance as inhibitors for the development of new cleaning chemicals for a green environment. Plant extracts are viewed as an incredibly rich source of naturally synthesized valuable chemical compounds that can be extracted by simple extraction procedures with low cost.

1.6. *Ficus*

The genus *Ficus* belongs to Moraceae family and constitutes more than 850 species which are distributed predominantly in the tropical and sub-tropical parts of the world (Pistelli *et al*, 2000). *Ficus* plants are traditionally used in folk medicine for the treatment of many illnesses such as convulsions and respiratory disorders (Wakeel *et al.*, 2004). The decoctions of *Ficus dekdekena* roots and *Ficus exasperata* stem barks are used in the treatment of gonorrhea (Kuete *et al.*, 2009). In Senegal, the maceration of the leaves of *Ficus dekdekena* is used to treat tuberculosis (Kuete *et al.*, 2009). *Ficus chlamydocarpa* and *Ficus cordata* are used traditionally in the treatment of filaris, diarrhoeal infections and tuberculosis (Khabe, 2007). The decoction from the mixture (1:1 w/w) of root bark from *Ficus* *chlamydocarpa* and stem bark of *Ficus cordata* are used in the treatment of oral infections (Khabe, 2007).

Plants from the genus *Ficus* are potential sources of flavonoids and isoflavonoids, lignans, terpenoids, alkaloids and coumarins (Kuo and Li, 1997, 2000; Amooru *et al*., 2005; Chiang *et al*., 2005). *Ficus carica* L. (the common fig plant) is one of the most important fruit trees cultivated in the Mediterranean coastal zone which grows well under calcareous soil conditions. The high percentage of calcium which constitutes about 35% of the mineral mass in the above-ground parts of fig trees may reflect the ability of fig trees to accumulate calcium (Abdel-Gaber *et al.*, 2008). A brief introduction of the *Ficus* species scrutinized in the present study for their antioxidant, antimicrobial and antiscalant activities is given below:

Ficus benglensis (Banyan tree) locally known as bargad is native to India, Srilanka and Pakistan (Amrit, 2006). In local medicine system, stem-bark, leaves and fruits are used to cure erysipelas, burning sensation and vaginal disorders, while an infusion of the bark cures dysentery, diarrhoea, leucorrhoea, menorrhagia, nervous disorders and reduces blood sugar in diabetes. Reports about the antioxidant, antimicrobial and about other bioactivities have been published (Manian *et al*., 2008).

Ficus infectoria syn. *F.virens* locally known as pakkar is used for the treatment of blood diseases, apoplexy, vertigo and delirium in ayurvedic medicine system. Abdel-Hameed (2009) conducted a study on the antioxidant activity of the leaves of *F.virens* from Egypt. ***Ficus racemosa*** (cluster fig) syn *F. glomerata* locally known as glur is distributed all over Pakistan. Different parts e.g. fruit, leaves, bark of this plant are used for the treatment of many diseases in local medicinal system (Chopra *et al*., 2002). It's therapeutic, antioxidant and antimicrobial activities have been reported (Rao *et al*., 2008, Verma *et al*.,2010).

Ficus religiosa (Pipal tree) known as peepal by the local community is the most popular member of the genus *Ficus*. The traditional systems of medicine used bark, fruit and leaves to cure different diseases like ulcers, gonorrhea, tuberculosis, asthma, cough, etc.***Ficus retusa*** **(**Chinese banyan tree) called chilkan locally is native to India, south China, and Austrailia (Wagner & Herbst, 1999). It's leave and roots are used as traditional medicine in India, Malaysia, and Southern China

(Hanelt *et al.,* 2001). Ao *et al.,* (2008) reported antioxidant and antimicrobial activities of this plant.

The use of synthetic antioxidant, antimicrobial and antiscalant agents is anticipated to create serious health hazards and environmental problems. The exploration of naturally occurring antioxidants, antimicrobials and antiscalants for food preservation, water purification and for other industrial usage is gaining much attention due to consumer awareness and consciousness about green chemicals (Schuenzel and Harrison, 2002). *Ficus* plants are viable source of high value components and useful secondary metabolites with multiple biological functionalities, however rarely investigations related to antioxidant and biological activities have been conducted on the important species of *ficus* commonly distributed in Pakistan. As the species of genus *Ficus* are widely distributed in Pakistan and are also easily available worldwide, hence the research on their antioxidant, antimicrobial and antiscalant attributes will be valuable towards exploring their potential utilization.

1.7. Detailed Aims and Objectives

The detailed aims and objectives of the present research are as follow

Screening of different physiological parts (fruit, leaves, bark etc) of selected species of *Ficus* as potential source of antioxidants.

Optimization of extraction procedures for recovery of effective antioxidant components by using different solvent systems and extraction techniques.

Evaluation of antioxidant activity of crude extract following different in-vitro antioxidant assays

Evaluation of antimicrobial and antiscalant activity of the extracts.

Characterization of selected bioactive compounds (phenolic acids and flavonoids etc.) by using state-of-the-art chromatographic/spectroscopic techniques.

CHAPTER-2

REVIEW OF LITERATURE

2.1. Plant Phenolics

Synthetic antioxidants which are routinely used as food additives because of their efficacy and efficiency, although are feasible to use on commercial basis, however, these have some toxic effects for humans (Shahidi and Wanasundara, 1992). On the other hand, based on the long term folk and therapeutic usage of the food plants, the natural antioxidants, especially the polyphenols, are recognized to be safe and healthier. Different types of phenolic compounds are present in the plants as secondary metabolites and on the basis of their basic carbon structure; they can be classified into thirteen (13) different classes (Shahidi and Naczk, 1995; Bravo, 1998; Manach *et al.,* 2004). Out of these naturally occurring phenolic compounds, phenolic acids and flavonoids are considered as the most important classes of compounds in plants (Kim *et al*., 2003; Yanishlieva and Maslarova, 2001).

2.1.1. Phenolic acids

The compounds in which a carboxylic functional group is directly attached to benzene ring are called phenolic acids (Lafay and Gil-Izquierdo, 2008). Phenolic acids are further structurally subdivided into two sub classes, the benzoic acid derivatives (C_6C_1) and the cinnamic acid derivatives (C_6-C_3) (Robbins, 2003). Different patterns of substitution (hydroxylation and methoxylation) on benzene ring produce different derivatives of benzoic acid and cinnamic acid. Mostly, these phenolic acid derivatives are found in plants as glycosides or coupled with different organic acids (quinic, shikimic, maleic and tartaric acids) through ester linkage (Herrmann, 1989, Shuster and Herrmann, 1985) or bounded with larger phenolic molecules (Winter and Herrmann, 1986; Klick and Herrmann, 1988; Clifford, 1999; Scalbert and Williamson, 2000). Vanillic acid, *p*hydroxybenzoic and gallic acid are common hydroxyl benzoic acids which are found almost in all plants (Shahidi and Naczk, 1995; Robbins, 2003). Caffeic, ferulic, *p*coumaric and sinapic acids are common naturally occurring cinnamic acid derivatives found in cereals fruits and vegetables (Andreasen *et al*., 2000; Scalbert and Williamson, 2000; Robbins, 2003).

2.1.2. Flavonoids

Among the phenolics, flavonoids are the most abundant compounds in plants contribute to almost 60% of the dietary phenolic compounds (Harborne and Williams, 2000; Shahidi and Naczk, 2004; Nichenametla *et al*., 2006) and more than nine thousand structures of flavonoids have been identified (Martens and Mithofer, 2005). In flavonoids basic skeleton constitutes 15-carbon atoms in the form of two aromatic rings which are linked through a three membered heterocyclic ring (C_6-C_3-C_6) (Martens and Mithofer, 2005; Mladenka *et al.*, 2010). The structure and numbering of carbon atoms in flavonoid basic skeleton is shown in fig 2.1. All the flavonoids are derived from this basic structure commonly known as 2-phenylchromane (Biesaga, 2011) by the addition of different substituents including benzyl, cinnamyl, hydroxyl, isoprenyl and methoxyl (Harborne and Williams, 2000). Anthocyanins, flavanols, flavanones, flavones, flavonols and isoflavones are the major subclasses of flavonoids but flavones and flavonols are the most abundant subclasses of flavonoids however, flavonols occur more frequently than flavones in plant tissues, particularly in leaves, flowers and fruits of higher plants (Harborne *et al.*, 1999). According to Seigler (1998) kaempferol, quercetin, rutin, catechin, epicatechin, myricetin, anthocyanidins and luteolin are the most common flavonoid aglycones isolated from the plants.

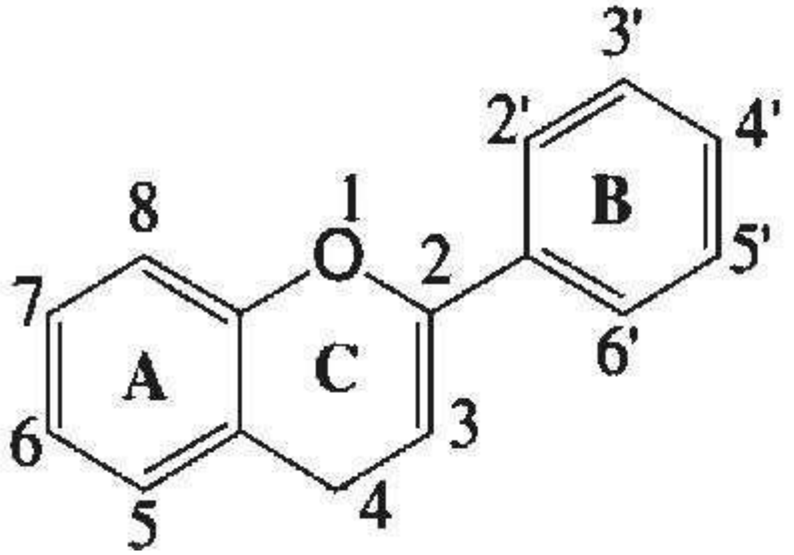


Figure 2.1. Structure of molecular nucleus of flavonoids

2.2. Structure and Antioxidant Activity of Phenolics

Phenolic molecules have diversity and unique features in structures possessing great potential against oxidative stress. According to Dai and Mumper (2010) antioxidant activity of phenolics and flavonoids is higher than that of vitamin C and vitamin E. They can exhibit their antioxidant activities through different mechanisms including free radical scavenging

(Rice-Evans *et al.*, 1996), oxygen scavenging and metal chelation (Heim *et al.*, 2002; Seyoum *et al.*, 2006). Antioxidant efficiency of plant phenolics depends on the number and position of hydroxyl groups on the phenyl ring (Aruoma, 2002; Erkan *et al.*, 2011; Mandal *et al.*, 2010). Higher number of hydroxyl groups on the benzene ring of phenolic acids and flavonoids results into higher antioxidant activity *in vitro* (Fukumoto and Mazza, 2000). According to Cuvelier *et al.*, (1992) antioxidant activity of benzoic acid derivatives is less than that of cinnamic acid derivatives because of unsaturation on side chain (-CH=CH-COOH) of the cinnamic acid derivatives which stabilizes the phenoxy radical through resonance (Marinova and Yanishleiva, 2003) while there is no unsaturation on the side chain (-COOH) of benzoic acid derivatives. De Heer *et al.*, (1999) described that hydroxylation at the ortho position of aromatic ring of phenolic compounds; thermodynamically favour the hydrogen bonding within molecule due to this property, phenolic compounds can donate hydrogen to free radicals easily. The number of free hydroxyl groups in the molecular structure of phenolic and flavonoid compounds enhances the reduction activity (Rice-Evans *et al.*, 1996). Fukumoto and Mazza, (2000) reported that glycosylation of quercetin, cyanidin, pelargonidin and peonidin resulted in lower antioxidant activity and the addition of a second glycosyl moiety decreased activity further. This decrease in antioxidant effect was attributed to steric hindrance by carbohydrate moiety. Yang *et al.*, (2001a) found that quercetin exhibited the highest antioxidant activity among eight flavonols studied. When the 3hydroxyl group of quercetin was glycosylated, as in rutin, the result was a significant decrease in antioxidant activity. The position and number of hydroxyl groups on the molecule of flavonoids has significant impact on the antioxidant activity of these compounds (Shahidi and Wanasundra, 1992). It is widely accepted that by increasing the number of hydroxyl groups on ring A and ring B of flavonoids can increase their effectiveness against the free radical reactions. Hydroxyl groups present on third and fourth carbon of ring B and the double bond between second and third carbon of ring C are the main cause of antioxidant activity of flavonoids (Chaillou and Nazareno 2006). Presence of ketone group on position 4 of ring C combined with the presence of hydroxyl group at position 3 of ring C or position 5 of ring A enables the flavonoids to act as metal chelators (Chaillou and Nazareno 2006; Frankel, 1999). But according to Rice-Evans *et al.*, (1996) metal chelating property of flavonoids varies from metal to metal. Burda and Oleszek (2001) investigated the relationship between the structure of 42 flavonoids and their antioxidant and antiradical activities. They reported that flavonols with a free hydroxyl group at the C-3 position of the flavonoid skeleton showed the highest inhibitory activity to β-carotene oxidation. Antiradical activity depended on the presence of a flavonol structure or free hydroxyl group at the C-4′ position. The effect of the 4′hydroxyl was strongly modified by other structural features, such as the presence of free hydroxyls at C-3 and/or C-3′ and a C2-C3 double bond.

2.3. Occurrence of Phenolics in Plants and *Ficus* species

Plants are considered as the richest source of phenolic antioxidants which are the secondary metabolites of plants (Dai and Mumper, 2010). Phenolic acids and flavonoids are the most abundant and widely distributed phenolics produced by plants (Apak *et al.*, 2007). A huge number of researchers and food scientists all over the world have shown keen interest in isolation and identification of phenolics including phenolic acids and flavonoids from thousands of medicinal plants including some *Ficus* species (Justesen *el al.,* 2001; Nguyen *et al.*, 2002; Hassan *et al.*, 2002; Wu *et al.*, 2002; Cai *et al.*, 2004; Atta and Abo El-Sooud, 2004; Dorman *et al.*, 2004; Li *et al.*, 2004; Chang *et al.*, 2005; Sannomiya *et al.*, 2005; Sheu *et al.*, 2005; Mavi *et al.*,2005; Lorenc-Kukula *et al.,* 2005;

Li *et al.*, 2006; Nahrstedt *et al.*, 2006; Teixeira *et al.*, 2006; Vaya and Mahmood, 2006; Velioglu *et al.,* 2006; Wojdyło *et al.*, 2007; Coruh *et al.*,2007; Silva *et al.*, 2007; Darbour *et al.*, 2007; Kogawa *et al.*, 2007; Yang *et al.,* 2008; Sultana and Anwar, 2008; Cheng *et al.,* 2008; Babu *et al.*, 2008; Al-Jaber, 2008; Akorum *et al.*, 2009; Chakroborty *et al.,* 2009; Miguel, 2009; Oliveira *et al.*, 2009; Niranjan *et al.*, 2010).The summary of phenolic acids and flavonoids identified form different *Ficus* species is presented in table 2.1.

Table 2.1. Summary of phenolics in different plant species

Family	Specie	Phenolic Components	Reference
Moraceae	*Ficus religiosa*	Myricetin, Quercetin, Kampeferol	Sultana and Anwar, 2008
	F.palmata	quercitin-3-glucoside, rutin	3
	F.benjamina	cinnamic acid , lactose , naringenin , quercetin , caffeic acid	Hassan *et al.*, 2002
	Ficus pumila	rutin , apigenin 6-neohesperidose , kaempferol 3-robinobioside and kaempferol 3-rutinoside	Cheng *et al.*, 2008

	F.carica	3-O- and 5-O-caffeoylquinic acids, ferulic acid, quercetin-3Oglucoside, chlorogenic acid, rutin, psoralen quercetin-3-O-rutinoside, psoralen and bergapten, quercetin, luteolin	Oliveira *et al.*, 2009; el-Kholy and Shaban, 1966; Teixeira *et al.*,2006; Vaya and Mahmood,2006
	F.septica	Genistin, Kaempferitrin, Vanillic acid	Wu *et al.*, 2002
	Ficus ruficaulis	Isoquercitrin, Rutin	Chang *et al.*, 2005
	Ficus formosana	Carpachromene, Isoglabranin, Norartocarpanone, Ficuformodiol A, Ficuformodiol B, Apigenin	Sheu *et al.*, 2005
	Ficus hirta	Apigenin, Hesperidin, 5-Hydroxy-4_,6,7,8-tetramethoxyflavone, 4_,5,6,7,8-Pentamethoxyflavone	Li *et al.*, 2006
	Ficus nymphaeifolia	Genistein, Alpinumisoflavone, Cajanin, 5,7,2_-Trihydroxy-4_methoxyisoflavone	Darbour *et al.*, 2007
	Ficus racemosa	Bergenin, Racemosic acid	Li *et al.*, 2004
	F. semicordata	(+)‒catechin, quercetin, quercitrin	Nguyen *et al.*, 2002

Ficus is one of the forty (40) genera belonging to family Moraceae (Woodland, 1997). According to Kislev *et al.*, (2006) the plants belonging to this genus are older more than 11 thousand years and this claim was supported by Gibbons, (2006). A large number of *Ficus* species is used in traditional folk medicinal system of India, China, Africa and in different countries of middle east. *Ficus* species are believed to be antioxidant, anticarcinogenic, anti-inflammatory and hepatoprotective and different parts (bark, fruit, leaves) of these species are used for the treatment of different diseases in local medicinal system. Many researchers analysed the *Ficus* species to find out their chemical constituents including phenolics to establish a relationship between claimed medicinal properties and phytochemicals present in the *Ficus* species (Ao *et al.*, 2008, Darbour *et al.*, 2007).

Sultana and Anwar (2008) investigated different vegetables, fruits, and medicinal plant organs including *F. bengalensis* fruit for their flavonol contents and found that *F. bengalensis* contained kaempeferol, quercetin and myricetin in notable concentrations. Other medicinal plants analysed included bark of *Acacia nilotica*, leaves of *Aloe barbadensis*, bark *Azadirachta indica,* roots of *Moringa oleifera* and bark of *Terminalia arjuna*. Quercetin and Kampeferol was found in all the samples under study while myricetin was not present in bark of *Azadirachta indica and Terminalia arjuna.* Hassan Abdalla Almatry, (2002) studied the *F.benjamina* fruit and bark and reported the presence of different phenolic components including quercetin, naringenin, caffeic acid and cinnamic acid.

Taskeen *et al.*, (2009) conducted a study on *Ficus bhengalensis* and *Ficus religiosa* for their flavonoids by using five standards (Kaempferol,rhamnetin, myricetin, isorhamnetin and quercetin). Only quercetin and myricetin were found in the samples analysed although quercetin was in higher amount than myricetin. They also concluded in their study that *Ficus bhengalensis* constituted higher concentration of flavonoids than *Ficus religiosa*. They did not mention in their report which part of the plant material was analysed however Subramanian *et al.,* (1978) reported that leaves of *F.benghalensis* contained Quercitin-3-glucoside and rutin. Twelve flavonoids (ten as aglycones and two as glycosides) including7,4´-dimethoxy-5-hydroxyisoflavone, 5,7,2´,5´-tetrahydroxy flavanone apigenin, chrysin, genistein, hesperitin, luteolin, naringenin, taxifolin , tricetin, rutin and isorhamnetin-3-glucoside were isolated from the aerial parts of *Ficus pumila* by Pistelli *et al.,*(2000). In an earlier study different flavonoid glycosides (astragalin, isoquercitrin, apigenin 6-*C*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside and kaempferol 3-*Oa*-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside) were isolated from *Ficus pumila* (Katajama *et al.,* 1998 a,b).

Veberic *et al*., (2008) investigated different cultivars of fruits of *F.carica*. Two of them (Cˇrna petrovka' and 'Miljska figa) were dark type fruit and one of them ('Zuccherina') was white type fruit. The phenolics extracted and identified from these fruits were gallic acid, chlorogenic acid, syringic acid, (+)-catechin, (-)-epicatechin and rutin. He concluded that fruits belonging two dark cultivars contained higher amount of phenolics in comparison with white fruit cultivar. They also reported that fruit samples collected from the second crop yielded higher phenolic contents than the fruit samples collected from first crop. Rutin was found in highest amount and syringic acid in lowest amount. The amount of rutin determined in fig fruits was comparable to that of apples.

2.4. Extraction of Phenolic Compounds from Plant Material

The composition of phenolics in plants extract is effected by many factors including pre-treatment of sample, polarity of the solvent applied for extraction (Sultana *et al.,*2009), ratio of the extraction solvent to the plant material, extraction technique, chemical nature of phenolic compounds present in the plant and interfering compounds (Naczk and Shahii, 2004). Typically, plant samples are air dried in most of the cases then meshed and frozen or kept at low temperature (4^0C) before applying extraction because phenolic compounds can undergo enzymatic degradation and are susceptible to polymerization during storage period (Price *et al.*, 1997). Phenolic compounds are not evenly distributed in plants e.g. these are in higher concentration in epidermal and subepidermal layers (outer tissues of plants) than in mesocarp and pulp (Antolovich *et al.*, 2000). Grinding of plant materials is used to resolve this problem leading to even distribution of phenolics before extraction . Moreover, extraction efficiency is increased due to increased surface area of the sample due to grinding (Sultana *et al.*, 2008; Wendakoon *et al.*, 2012).

Solubility of phenolic compounds is different in different solvents due to their structural diversification. Due to this limitation, extraction of phenolic compounds from plant material is largely dependent on the type of solvent. Polarity of the solvent affects the solubility of phenolic compounds (Harborne and Williams, 2000; Shahidi and Naczk, 2004; Zubair *et al.*, 2012). Several organic solvents like ethanol, ethyl acetate, methanol, acetone and their aqueous combinations have been used for the extraction of phenolic compounds (Robbins, 2003; Miliauskas *et al.*, 2004; Rajeshwar *et al.*, 2005; Djeridane *et al.*, 2006; Adesegun *et al.*, 2007; Arabshahi-Delouee and Urooj 2007; Dukic *et al.*, 2008; Potchoo *et al.*, 2008; Sultana *et al.*, 2009; Rohman *et al.*, 2010; Manzoor *et al.*,2012). However, manufacturers of herbal medicine used ethanol frequently as extraction solvent due to its non-toxic effects and biodegradable nature (Low Dog, 2009). Moreover, ethanol and water concentrations employed in the extraction process also affect the bioactivity of plant extracts (Ganora, 2008). Aqueous methanol solutions, specifically, are one of the most commonly employed solvents for extracting phenolic compounds, particularly phenolic acids and flavonoids, from fruit, vegetable and other plant materials (Merken and Beecher, 2000) since phenolic compounds are quite stable in these methanol solutions. For example, flavones and flavonols were reported to be stable in methanol for greater than three months at 4°C (Hertog *et al.*, 1992b). Aqueous methanol solutions also result in higher extraction yields of phenolic acids and flavonoids.

El-Sayed *et al.*, (2009) conducted a study to evaluate the total phenolic contents and antioxidant activities from the leaves of *Ficus sycomorus* and *Azadirchta indica* by using methanol, methanol-water mixtures and water for the extraction of bioactive components from these plants. In this study extraction yield of the *Ficus sycomorus* leaves was improved from 4.06 (for pure water) to 14.30 (for 70% aqueous methanol) and similar results were obtained for the leaves of *Azadirchta indica*. Metivier *et al.* (1980) reported that aqueous methanol was 73% more effective than water and 20% more effective than aqueous ethanol solution of the same concentration in extracting anthocyanins from grape pomace. Although most of the literature confirmed that aqueous mixtures of methanol or ethanol give higher yields and better antioxidant activity in comparison with absolute methanol or ethanol but certain number of instances are also reported when the extraction yields and antioxidant activity of the extracts obtained by the pure solvent was better than the extracts obtained with their aqueous versions. Nazzaro *et al.*, (2012) reported that the values of extraction yield and total polyphenol content obtained from *Corylus avellana* L. decreased according to the extraction solvent in the following order: absolute methanol > 70% methanol > water. Sultana *et al.*, (2009) also reported that *Eugenia jambolana* bark and *Terminalia arjuna* bark produced higher extraction yield when absolute methanol was employed for extraction in comparison to 80% methanol. They also reported that barks of *Eugenia jambolana*, *Acacia nilotica*, *Azadirachta indica* and *Terminalia arjuna* gave higher total phenolic contents and total flavonoid contents with absolute methanol instead of 80% methanol. Extraction of phenolic constituents from plants is also greatly affected by the solvent to plant solid ratio. In another study to evaluate the effect of solvent to solid sample ratio on canola meal by using aqueous acetone, the solvent to solid sample ratio was varied from 5:1 to 10:1 and this variation increased the quantity of total phenolics from 773 to 805 mg from 100 g of sample. Efficiency of extraction method also influenced by the temperature and duration (Me *et al.*, 2007). By increasing temperature, extraction yield of phenolic contents also increased. Yield with hot solvent is higher than that with cold solvents (Katalinic *et al.*, 2006). Duration of extraction varies depending on the temperature and technique used. At higher temperature duration of extraction is decreased. In literature duration of extraction is reported from sixty seconds (Sun *et al.*, 2001) to one day (Maxson and Rooney, 1972) depending upon the extraction process employed. Single extraction is not usually enough to extract all available contents of extractable components, hence, often, for optimum extraction of phenolic components, process of extraction is repeated two to three times and then the three extracts are mixed (Merken and Beecher, 2000; Robbins, 2003). More than three repetitions have marginal effect on the extraction yield (Shahidi and Naczk, 2004).

Selection of an appropriate and effective technique is also important to recover maximum amount of extractable phenolic compounds from plant matrices. Typical techniques used for this purpose include reflux, soxhlet and maceration (Kelen and Tepe, 2007; Bhalodi *et al.*,2008; Motlhanka, 2008; Vaidya *et al.*, 2008; Chahardehi *et al.*, 2009; Jayakumar *et al.*, 2009; Sharififar *et al.*, 2009; Tian *et al.*, 2009; Rohman *et al.*, 2010). Veerapur *et al.*, (2009) applied soxhelt extraction technique for the extraction of phenolic compounds from stem bark of *F. racemosa* by using 95% ethanol and water as solvents. Sultana *et al.*, (2009) compared the efficacy of extraction procedure for recovering phenolic components from *F. religiosa* fruits by applying four solvents (absolute ethanol, absolute methanol, 80% ethanol and 80% methanol) and two techniques (orbital shaker and reflux). They reported that 80% aqueous-methanol in combination with orbital shaker technique yielded the highest quantity of phenolic components as compared to 80% aqueous-ethanol, absolute ethanol and absolute methanol solvent systems and reflux technique. Other modern techniques reported for the extraction of phenolic compounds include super critical fluid extraction SCFE), pressurized fluid extraction, sonication and microwave assisted extractions (Rafeal *et al.*, 2008; Dai and Mumper, 2010; Zubair *et al.*, 2012).

Ultrasound-assisted extraction is relatively economic technique as compared to others (e.g. super critical fluid extraction) and requires simple instrumentation like ultrasonic bath (Vinatoru, 2001). In recent years applications of ultrasound-assisted extraction for the extraction of phenolic components from different parts (fruit, leaves, stalk) have been studied (Montiel-Herrera *et al.*, 2004; Yang *et al.*, 2008; Paniwnyk *et al.*, 2009). Solubility of phenolic components from plant matrix into extracting solvent is also an important parameter which can contribute towards affecting the extraction yield (Shon *et al.*, 2004; Sultana *et al.*, 2009).

2.5. Measurement of Antioxidant Activity

Various *in vitro* and *in vivo* experimental protocols have been developed so for to evaluate the antioxidant activity of plant extracts. Phenolic compounds act as antioxidant through different mechanisms e.g. by metals chelation, singlet oxygen quenching, reducing different molecules and ions and deactivating free radicals by donating hydrogen to them. Due to complex nature of extracts, no single method can be applicable to fully and truly evaluate the antioxidant activity of plant extracts. Hence different assays are used for the measurement of antioxidant activity of extracts. Antioxidant assays can be divided in to two categories

(a) Hydrogen Atom Transfer (HAT) reaction based assays: HAT-based assay system commonly constitutes an oxidizable molecular probe, a synthetic free radical generator and an antioxidant. These assays are related to radical chain breaking capacity of an antioxidant and evaluate the free radical scavenging capacity of an antioxidant by donating a hydrogen atom.

(b) Single Electron Transfer (ET) reaction based assays: Electron transfer based assays constitute an oxidant and an antioxidant in the reaction mixture. The oxidant which is also called probe is reduced by the antioxidant after the transfer of electron from antioxidant to prob. After reduction, probe changed its colour and the intensity of colour change is directly proportional to the concentration of antioxidants. When the reaction is reached to its end point then colour change stops. The change in colour is noted at different concentrations of antioxidant by taking absorbance through spectrophotometer. Then a graph is plotted between absorbance and concentration of antioxidant. Concentration is taken on x-axis and absorbance on y-axis. Through the regression equation slope of the curve is determined which is equivalent to reducing capacity of antioxidant which is expressed as Trolox equivalence or Gallic acid equivalent. The main limitation of this reaction is that there is no competitive reaction involved in this reaction due to which it is questionable to relate the results with total antioxidant capacity (Huang *et al.*, 2005).

All the tests listed below in the table 2.2 are *in vitro* tests. Evaluation of the potential antioxidant activity of a phenolic compound or extract typically begins with *in vitro* tests for free radical scavenging (Aruoma, 2002; Sanchez-Moreno, 1999). Compared to *in vivo* methods, *in vitro* analysis is generally lower in cost, faster and may provide mechanistic information on the antioxidant by testing various types of free radicals and/or reactive oxygen species. Compounds that have poor antioxidant activity *in vitro* will not be effective *in vivo* or in a food system (Aruoma *et al.*, 1997). Effective antioxidant activity *in vitro*, however, does not necessarily indicate that the same compound will be effective *in vivo* since the *in vitro* test systems cannot effectively simulate the complex metabolism of the human body.

Table.2.2. Different *in-vitro* antioxidant assays used for activity evaluation of antioxidants

S.No.	Name of Assay	Reference

(a)	**Hydrogen Atom Transfer Methods (HAT)**	
1)	Crocin bleaching Nitric oxide radical inhibition activity	Bors *et al.*, 1984
2)	Inhibited oxygen uptake (IOC)	Burton, 1981
3)	Lipid peroxidation inhibition capacity (LPIC) assay	Kleinveld *et al.*, 1992
4)	Oxygen radical absorbance capacity (ORAC) method	Cao and Prior, 1999
5)	Total radical trapping antioxidant parameter (TRAP)	Wayner *et al.*, 1985
(b)	**Electron Transfer Methods (ET)**	
1)	Copper (II) reduction capacity	Zaporozhets *et al.*, 2004
2)	DPPH free radical scavenging assay	MacDonald-Wicks *et al.*, 2006
3)	Ferric reducing antioxidant power (FRAP)	Benzie and Strain, 1996
4)	Total phenols by Folin-Ciocalteu	Singleton *et al.*, 1999
5)	Trolox equivalent antioxidant capacity (TEAC)	Miller *et al.*, 1993

2.5.1. Total Phenolics Assay

For the overall and speedy estimation of the phenolic contents from natural extracts, Folin–Ciocalteu reagent based spectrophotometric method is preferred and used by most of the researchers (Huang *et al.*, 2005; Khanizadeh *et al.*, 2008b; Lachman *et al.*, 2003; Anwar *et al.*, 2009; Li *et al.*, 2009; Pawar, Pai, Nimbalkar, and Dixit, 2011; Zubair *et al.*,2012). Initially this method was developed by Folin and Ciocalteu for the analysis of tyrosine in 1927, then Singleton and Rossi (1965) applied this method for the determination of total phenols after that it is applied for the estimation of total phenols and reported by many researchers (Anwar *et al.*, 2009; Sultana *et al.*, 2009; Zubair *et al.*, 2012). Singleton and Rossi (1965) tried to standardize the method by outlining different steps to avoid inconsistency in results. This test is more specific to phenolic compounds if carried out in alkaline medium and measurement of absorbance at 765 nm minimizes the interference from sample matrix (Singleton and Rossi, 1965). Although chemistry behind this reaction is not well defined but it is widely accepted that molybdenum present in Folin–Ciocalteu reagent gains electron from phenolic compounds present in plants extract and in this way molybdenum (Mo) reduces from Mo(VI) to Mo(V) and a blue complex is formed (Magalhaes *et al.*, 2008).

Total phenolic contents of the *Ficus* species and other medicinal plants are widely determined by using Folin–Ciocalteu reagent. Debib *et al.*, (2013) estimated the total phenolic contents from the dried fruits of two Algerian varieties (Azendjar and Taamriout) of *Ficus carica L.* with the help of Folin–Ciocalteu reagent and total flavonoid contents by using aluminium chloride method and found that aqueous extract and acetone extract contains higher concentrations of phenolic compounds and flavonoids. Shi *et al.*,(2011) determined the total phenolic contents by using Folin– Ciocalteu reagent and total flavonoid contents by using aluminium chloride method from the leaves of seven *Ficus* species including *F. virens var. sublanceolata, F. auriculata, F. vasculosa, F. callosa, F. virens var. verins, F. racemosa and F. oligodon* and concluded that *F. virens var. sublanceolata* have the highest contents of total phenolics and total flavonoids among all the tested species.

2.5.2. DPPH Radical Scavenging Capacity Assay:

DPPH is much popular assay for evaluation of radical scavenging activity than other assays used for this purpose and is widely used to evaluate plants antioxidant activity(Pereira *et al.*, 2006; Ferreira *et al.*, 2007; Sousa *et al.*, 2006). This test was first described by Blois in 1958 and then adopted by many researchers with changes and modifications according to samples under trial (Anwar *et al.*, 2009; Ferreira *et al.*, 2007; Pereira *et al.*, 2006 ; Sousa *et al.*, 2006; Sultana *et al.*, 2008; Zubair *et al.*, 2012). DPPH, a stable free radical, which is commercially available and there is no need to generate it during the assay. DPPH produces deep purple colour in free form and on reduction by an antioxidant is converted into a hydrazine with pale yellow colour. Free radical scavenging activity of a sample is observed by monitoring the decrease in absorbance of DPPH solution after the addition of sample with the help of spectrophotometer at wavelength of 515-528 nm. Generally, the radical scavenging activity of sample is expressed as IC_{50} value which is the concentration of the sample in the reaction mixture which can reduce DPPH concentration by 50% from initial level. According to Ozecelik *et al.*,(2003), after the reaction of DPPH with antioxidant, the absorbance of the reaction mixture also decreased by light, solvent type and oxygen(Apak *et al*., 2007), hence the absorbance of the DPPH must be inferred with great care. Previously it was considered that reaction between antioxidant and DPPH occurred due to transfer of hydrogen but recent research suggested that it is based on electron transfer reaction (Fotti *et al*., 2004; MacDonald-Wicks *et al*., 2006). According to kinetic study, the rate determine step is very fast because electron transfer takes place in this step and this is followed by transfer of hydrogen which depends on the solvent ability to accept the neutral hydrogen bond (Huang *et al*., 2005).

Ao *et al.,* (2008) investigated radical scavenging activity of bark, fruit and leaves of *Ficus microcarpa* L. by using DPPH assay and found that bark and fruit extract of the sample did not exhibit significant difference for their radical scavenging activity whereas the lowest scavenging activity observed for leaves. The aqueous extracts of *Ficus asperifolia* and *Gossypium arboretum* were tested for their DPPH radical scavenging activity wherein *Gossypium arboretum* exhibited significantly higher activity than that of *Ficus asperifolia* (Annan and Houghton, 2008).

2.5.3. Evaluation of Reducing Power

Antioxidant activity of plant extracts can be evaluated efficiently by measuring their reducing power (Ksouri *et al*., 2008). Reducing agents such as phenolic compounds can react with free radicals to stop their reactivity and in this way higher reducing activity of plant extract mean higher antioxidant activity (Hsu *et al.,* 2006). Different protocols have been developed for measuring the reducing power of botanical extracts. One of these which is widely used and reported is ferric reducing antioxidant capacity (FRAP) assay. FRAP assay is simple, rapid, inexpensive, and robust and does not require specialized equipment. The FRAP assay can be performed using automated, semiautomatic, or manual methods (Huang and Prior, 2005). Through this assay we measure the capability of plant extract to convert ferric tripyridyltriazine complex to its ferrous form which is accompanied by change in colour from yellow to greenish blue (Sousa *et al*., 2008). The resulting colour is measured with spectrophotometer and the intensity of colour is assumed to be proportional to the antioxidant activity of the extract i.e. higher the intensity higher is the antioxidant activity (Benzie *et al*., 1999; Huang *et al*., 2006). Any electron-donating substance even without antioxidant properties with redox potential lower than that of the redox pair Fe(III)/Fe(II) can contribute to the FRAP value and indicate falsely high values (Huang and Prior, 2005). Another protocol related to this assessment was initially described by Oyaizu

(1986). In this assay, substances having antioxidant activity converts potassium ferricyanide (Fe^{+3}) in to potassium ferrocyanide (Fe^{+2}) and ferric chloride to ferrous chloride in phosphate buffer (pH 6.6). In this way coloured complex formed has its λ_{max} at 700 nm, higher the absorption of reaction mixture means higher reducing power (Huang and Prior, 2005).

Thingbaijam *et al.* (2012) conducted a study to evaluate the reducing power of leaves of *F. Auriculata* L.by using potassium ferricyanide- ferric chloride system. He used different concentrations of the sample from 0.2-1.0 mg/mL in the reaction mixture and concluded that reducing power of the sample increased by increasing the concentration of sample in reaction mixture. Anandjiwala *et al.,* (2008) evaluated the reducing power of stem barks of *F.bengalensis, F.glomerata, F.religiosa* and *F.virens* using potassium ferricyanide-ferric chloride system and found that by increasing the concentration of sample in reaction mixture reducing power also increased. He also reported that 400µg of all the samples showed maximum reducing power.

2.5.4. Stabilization of Linoleic acid

Stabilization of linoleic acid system by using extracts from botanical sources is widely used for the evaluation of antioxidant activity of plant extracts (Siddhuraju and Becker, 2007; Anwar *et al.*, 2009a; Hussain *et al.*, 2010). In this assay, linoleic acid acts as substrate in acidic buffer (ethanol-phosphate) and on peroxidation produces a red coloured complex by reacting with ferric thiocynate. The absorbance of red colour developed is measured at 500 nm. The addition of plant extract delayes the peroxidation of linoleic acid and the intensity of red colour is reduced. Sultana *et al.,* (2007) evaluated the inhibitory effect of the bark extracts of *Azadirachta indica, Terminalia arjuna, Acacia nilotica*, and *Eugenia jambolana* Lam by stabilizing the linoleic acid system. They concluded that all the bark extracts have excellent inhibitory effect on the peroxidation of linoleic acid which was comparable to those of standards (BHA, BHT and PG) used as positive control. Shi *et al.,*(2011) also reported the inhibitory effect of the extracts from the leaves of seven *Ficus* species (*F. virens var. sublanceolata, F. auriculata, F. vasculosa, F. callosa, F. virens var. verins, F. racemosa and F. oligodon*) on the peroxidation of linoleic acid. According to their research the inhibitory effect of different species was in order of *F. virens var. sublanceolata, F. callosa, F. auriculata, F. virens var. verins* and *F. oligodon*. All these five species exhibited appreciable inhibition of linoleic acid peroxidation.

2.6. Chromatographic characterization of phenolic compounds:

Separation, purification and identification of phenolic compounds can be achieved by applying different chromatographic techniques (Merken and Beecher, 2000; Robbins, 2003; Shahidi and Naczk, 2004 Shahidi and Naczk, 2004) including paper chromatography (PC) (Haslam, 1996; Jackman *et al.*, 1987), thin-layer chromatography (TLC) (Mabry *et al.*, 1970; Azar *et al.*, 1987; Ahoua *et al.*, 2012), liquid column chromatography (Salagoity-Auguste and Bertrand, 1984; Fulcrand *et al.*, 1999), gas chromatography (GC) (Dabrowski and Sosulski, 1984; Liggins *et al.*, 1998; TasioulaMaragari and Okogeri, 2001) and, high performance liquid chromatography (HPLC) (Merken and Beecher, 2000; Proestos *et al.*,2006; Albayrak *et al.*, 2010). HPLC is used most frequently for the isolation and identification of phenolics (Merken and Beecher, 2000; Maatta et al., 2003; Robbins, 2003; Sultana *et al.*, 2008; Jinling *et al.*, 2012). Many factors are important during isolation and quantification of phenolic compounds with HPLC. These factors include preparation of sample solution, choice of solvent system for elution, Choice of column and detector.

Phenolic compounds are present in plant mostly as glycosides or esters or bounded to cell wall and they rarely occur as aglycone. To convert the glycosides and bounded form of phenolic compounds in to free form many researchers used the method of hydrolysis (Jinling *et al.*, 2012; Sultana *et al.*, 2008; Pereira *et al.*, 2007). Acidic, alkaline and enzymatic are three modes of hydrolysis frequently used but acidic and alkaline hydrolysis are preferred methods to convert glycosides and bound form of phenolic acids and flavonoids in to their respective aglycones (Arranz *et al.*, 2009; White *et al.*, 2010).

Sodium hydroxide aqueous solutions having concentration range from 1 to 4 *M* are usually used for basic hydrolysis of phenolic components in plants. Usually basic hydrolysis is done at room temperature but the time varies from fifteen minutes to twenty four hours. Usually the concentration of phenolic aglycones increased with increase of time for hydrolysis (Bonoli *et al.*, 2004). For acidic hydrolysis, any mineral acid in organic solvent can be used but HCl is the most common mineral acid used for this purpose while methanol is used frequently as organic solvent for acidic hydrolysis of plant materials. An antioxidant like BHT, TBHQ or ascorbic acid is also added during the hydrolysis of plant material to save the plant phenolics from degradation. Different concentrations of HCl in methanol are reported in the literature. Sultana and Anwar (2008) used 1% HCl in methanol to hydrolyse the phenolic components from different plant materials including the fruits of *Ficus religosia* and also used TBHQ as antioxidant. Huang *et al.*, (2006) used 6 M methanolic

HCl solution and refluxed this with samples from different plants for two hours on water bath for the preparation of aglycone samples for HPLC analysis.

Reverse phase (RP) HPLC remains the choice of researchers for isolation and characterization of plant phenolics (Snyder *et al.*, 2010) due to its various advantages over normal phase HPLC. In reverse phase HPLC stationary phase i.e. column is non polar while mobile phase is polar. Hence different strengths of polarities can be achieved by changing the composition of mobile phase and a variety of mixtures or natural extracts can be separated by using different compositions of mobile phase with same column. In this type of chromatography, analyte molecules are separated on the basis of hydrophobicity and partitioned themselves between non-polar stationary phase and polar mobile phase and retention time of non-polar components is greater than that of polar components (Meyer 2010, Snyder *et al.*, 2010). In most of the cases mobile phase is based on two solvent systems, conventionally named as solvent A and solvent B. Solvent A, usually consists of pure water or acidified water and commonly organic acids (e.g. acetic acid, trifluoroacetic acid) are used for this purpose. Solvent B is organic in nature which may be a pure organic solvent or mixture of organic solvents. Methanol and acetonitrile are the most common organic solvents used for the separation of phenolic compounds (Snyder *et al.*,2010). Sultana and Anwar (2008) used 3% trifluoroacetic acid aqueous solution as solvent A and a mixture of acetonitrile and methanol (80:20 v/v) was used as solvent B for HPLC analysis of flavonols from the fruits of *F.benglensis* and other plant materials.

Column has a pivotal rule in the HPLC analysis as it is the place where separation of mixture components occurs. Most of the researchers reported the use of reversed phase columns with 250 mm length, 4.6 mm internal diameter and 5 µm particle size (Tokusoglu *et al.*, 2003; Gorinstein *et al.*, 2004; Vrchovska *et al.*, 2006; Dragovic-Uzelac *et al.*, 2006; Wang *et al.*, 2007). However use of column with length of 150 mm has also been reported (Huang *et al.*, 2006, Klimczak *et al.*, 2006). Although alumina, graphitised carbon, silica, titanium, and zirconium are used in RP-HPLC column packing but silica is preferred over other supports because of its higher efficiency. Its particles are stable against higher pressure due to their mechanical strength. However zirconia-based columns are preferred where separations are done on higher temperature due to their higher thermal stability (Cacciola *et al.*, 2007a; Snyder *et al.*, 2010).

In HPLC instrument next to the column, detector is placed. Different types of detectors including refractive index-, fluorescence-, conductivity-, amphoteric-, chiral- and ultraviolet-visible (UV-VIS) detectors are available commercially in the market but most of the researchers reported UV-VIS detectors for the analysis of phenolic compounds (Wang *et al.* 2007; Surveswaran *et al.*, 2006; Klimczak *et al.*, 2006; Huang *et al.*, 2006; Dragovic-Uzelac *et al.*, 2006). UV-VIS detectors are available in three different types (fixed wavelength, variable wavelength and photodiode-array detectors) but photodiode-array detectors are the most popular among these three types of UV-VIS detectors because of their ability to analyze the absorbance of each analyte on different wavelengths during single chromatographic run and in this way optimal wavelength can be selected for HPLC analysis.

Phenolic compounds absorb radiations in the range of 190-700 nm (UV-VIS) due to the presence of aromatic ring and C-C double bond. Derivatives of hydroxyl benzoic acid exhibit their λ_{max} between 200 nm to 290 nm (Sharma *et al.*, 2005). Due to additional C-C double bond conjugation, derivatives of hydroxyl cinnamic acid derivatives have their λ_{max} in the range of 270 nm to 360 nm (Stalikas, 2007). But according to AbadGarcia *et al., (*2009a) the range for hydroxyl benzoic acid derivatives is from 255 nm to 280 nm and that of for cinnamic acid derivatives is from 310-325 nm. Although both classes of phenolic acids have wide range of absorption but most of the researchers reported 280 nm for the detection of phenolic acids of both classes (Klimczak *et al.*, 2007; Huang *et al.*, 2006; Russel *et al.*, 2009). For the detection of flavonoids this range extends from 350 nm to 370 nm and has been reported by most of the authors (Ledda *et al.*, 2010; Sultana and Anwaer 2008;).

The most common and the easiest method to identify the peaks in a chromatogram of a sample is the comparison of retention time of different peaks in a chromatogram with that of a standard compound under similar chromatographic conditions. If the retention time of a peak in the chromatogram of sample is same as that of a standard compound then both the substances may be similar. However, it is important to perform some more experiments to increase the degree of certainty. For HPLC, qualitative analysis can be improved without much expenditure e.g. to improve the degree of certainty we can compare absorbance ratio of standard and that of sample because absorbance ratio between two random wavelengths is compound specific. If the sample and the standard are the same substance then the increase or decrease in the signal of sample and standard should be of same degree.

For quantitative analysis, a calibration graph is developed with at least three data points. Calibration can be performed by using three different methods: external standard, internal standard and standard addition but the external standard method is the most common method for determination of concentration of an unknown sample. In this method, solutions of the reference compound with different concentration are prepared and equal volume of these standard solutions are analysed by HPLC under same conditions used for the analysis of sample. Then calibration graph is plotted between concentrations and peak responses. The range of concentrations for standard solutions must be chosen carefully so that calibration graph obtained must be linear and the concentration of sample must also fall within this range.

This technique has been used for the study of bioactive tannins, flavonoids and their derivatives in different plant preparations (Wei *et al*., 2011; Lou *et al*., 2010). Veberica *et al.*,(2008) analysed the fruits of *Ficus carica* for their phenolic acid and flavonoids components through RP-HPLC. He used 0.01 *M* phosphoric acid as solvent A and Methanol (100%) as solvent B. Phenomenex Synergi 4u MAX – RP 80 A column was used and mobile phase flow rate was 1 mL/min. The detection was carried at a wavelength of 280 nm using a PDA detector. The compounds detected from the sample include chlorogenic acid, gallic acid, syringic acid, catechine and rutine. Sultana and Anwar (2008) identified kaempeferol, quercetin, myricetin from the fruits of *Ficus religiosa* through RP-HPLC equipped with Supelco ODS (C18) column (250 X 4.6 mm; 5 µm particle size) at a wavelength of 360 nm using UV-VIS detector.

Vallejo *et al.*, (2012) studied eighteen varieties of *Ficus carica* from south-eastern Spain on Hitachi LC-DAD (L-2455) equipped with Merck LiChrospher ODS-18 column (250 X 4 mm; 5 µm particle size). 5% aqueous formic acid was used as solvent A and 100% methanol was used as solvent B. They used gradient elution and chromatograms were recorded at 280, 320, 360 and 510 nm. The important phenolic compounds identified include Chlorogenic acid, Kaempferol-rutinoside, Quercetin-rutinoside and Cyanidin-3-rutinoside.

Ao *et al*., (2008) identified phenolic compounds from the bark of *F. microcrapa* with the help of HPLC at a wavelength of 280 nm. Aqueous acetic acid (0.5%) was used as solvent A and 25% acetonitrile in methanol was used as solvent B. Gradient elution was performed using Synergi 4u Hydro-RP 80A column (150 mm X 4.60 mm, Phenomenex Company,USA). Seven phenolic compounds including protocatechuic acid, catechol, p-vinylguaiacol, syringol, p-propylphenol, vanillin and syringaldehyde were quantified in ethyl acetate fraction of bark by HPLC.

In recent years hyphenated techniques are getting popularity for the detection of phenolic compounds from the extracts of plants. In hyphenated techniques HPLC is coupled with another analytical instrument like mass spectroscope (MS), Fourier transform infrared (FTIR) spectroscope and Nuclear Magnetic Resonance (NMR) spectroscope which serves as detector. But out of these, HPLC-MS is widely used for the detection of phenolic compounds from plant extracts. MS is coupled with HPLC through an interface where mobile phase is evaporated and the analyte is converted in to ions. There are different types of interfaces available including atmospheric pressure ionisation, fast atomic bombardment, electron spray ionisation, laser desorption and thermospray but atmospheric pressure chemical ionisation (APCI) and electron spray ionisation (ESI) are the most popular among these. Generally ESI is used for thermolabile molecules and is also known as soft ionisation technique. On the other hand, APCI is used for stable and non-polar molecules and not suitable for thermolabile compounds as harsher ionisation conditions are used which increase the risk of analyte degradation. After ionisation, sample ions are transferred to mass analyser , although different types of analysers like ion trap, quaderpole, time of flight and magnetic and electrostatic sector have been developed, but their basic purpose is to separate ions on the basis of their mass to charge (m/z) ratio and move these ions in to electron multiplier for detection.

Table 2.3. Summary of different HPLC conditions used for the separation of phenolic compounds from different botanical sources

Compounds	Plant sample/material	Column	Solvent system	Detection	Reference
Catechines	*Acacia confuse* (Stem Bark)	Hypersil ODS (250 mm × 4.6 mm× 2.5 µm)	A: water containing 0.5 % trifluoroacetic acid; B: acetonitrile containing 0.5% trifluoroacetic acid; Flow rate: 1 mL/min;	ESI-MS	Wei *et al.*, 2011
Phenolic acid and flavonoids	*Arctium lappa* (Leaves)	BEH C18 (150 mm ×2.1 mm × 1.7 µm)	A: water containing 0.1% formic acid; B: acetonitrile/methanol (20/80); Flow rate: 0.28 mL/min;	PDA, ESI-MS-MS	Lou *et al.*,2010
Flavonoids	*F. bengalensis*, *F. Religiosa*	(250 mm × 4.6 mm ×5 µm)	acetonotrile/water 1:1 , flow- rate of 1 mL min-1	UV-VIS, 254 nm	Taskeen *et al.*, 2009
Phenolic acid and flavonoids	*F. carica* fruit	Synergi 4u MAX – RP 80 A	A: Aqueous 0.01 M phosphoric acid; B:100% methanol; flow rate of 1.0 ml/min	DAD, 280,350 nm	Vebrica *et al.*, 2008
Flavonoids	*F. carica* fruits	Symmetry C-18 (150 mm × 2.1 mm ×3.5 µm)	A: water, acidified with 0.3% formic acid; B: methanol acidified with 0.3% formic acid; flow rate of 0.2 mL per min	UV–VIS photodiode Array, 370 nm	
Phenolic acids	*F. carica* leaves	Zorbax Eclipse XDB-C18 (250 mm × 4.6 mm ×5 µm)	A: acetonitrile; B: water with acetonitrile (2.5%) and formic acid (0.5%) flow rate 1.0 mL min^{-1}	DAD,	Teixeira *et al.*, 2006
Phenolic acids	*F. glomerata*	Phenomenex Luna RP, C 18 column (150 mm× 4.6 mm ×5 µm)	A: water containing 1% acetic acid; B: acetonitrile: flow rate of 1.0 ml/min	UV detector SPD – 10A, 280 nm	Verma *et al.*, 2010

Phenolic compounds	*F. microcarpa*	Synergi 4u Hydro-RP 80A column (150 mm× 4.6 mm ×5 μm) Phenomenex Company, USA)	A:water:acetic acid, 100:0.5, v/v; B: methanol:acetonitrile,3:1, v/v; flow rate of 0.8 ml/min	UV-VIS, 280 nm	Ao *et al.*,2008
Flavonol	*F. religiosa* fruits	Supelco ODSC18(250 mm × 4.6 mm ×5 μm)	A: contained 3% trifluoroacetic acid; B: contained acetonitrile and methanol (80:20 v/v); flow rate 1.0 mL min^{-1}	UV-VIS, 365	Sultana *et al.*, 2008
Flavonoids	*Juglans regia* L.	LiChro CART RP C18 (250 mm × 4 mm × 5 μm)	A: water containing 0.1% TFA; B: methanol; Flow rate: 1 mL/ min	PDA, MS-MS	Jalili and Sadeghzade, 2012
Flavonoids	*Maytenus aquifolium* and *Maytenus ilicifolia* Leaves	Supelcosil C8 and C18 (250 mm × 4.6 mm ×5 μm)	A: water containing 2.0, 2.5 or 3.0% formic acid or 0.3% trifluoroacetic acid; B: acetonitrile or methanol; Flow rate 1.0 mL/ min	PDA,	Diagone *et* al., 2012
Hydroxycinnamic acids, Flavonol glycosides	*Prunus armeniaca* (fruit)	250 × 4.6 mm, 5 μm Pinnacle-II C18	A: 3% acetic acid in water B: water/acetonitrile/acetic acid (73:25:2, v/v/v).	PDA, 278 nm	Dragovic-Uzelac *et al.* (2006)
Phenolic acid and flavonoids	*Prunus armeniaca* (fruit)	Gemini C18 (150 mm × 4.6 mm × 3 μm)	A: citric acid (75 mM); B: ammonium acetate (25 mM); Flow rate: 1.0 ml/min;	UV-VIS,	Zitka *et al.*, 2011
Phenolic cmpounds	Indian medicinal plants	250 × 4 mm, 5 μm Nucleosil 100-5 C18	A: 2.5 % formic acid B: methanol	PDA 280, 320, 420, 520 nm	Surveswaran *et al.*, 2007

2.7. Antimicrobial activity

2.7.1. Antimicrobial activity of plant phenolics

It is widely accepted that different bioactivities of plants including antimicrobial activity are because of secondary metabolites present in plants as a part of their defence system (Kalimuthu *et al.*, 2010). Compounds having antimicrobial activities in plants are subdivided in to two groups as phytoanticipins and phytoalexins. Phytoanticipins are stored in the plant cells as a proactive defence system of plant against microorganisms. Phenolic glycosides are the example of phytoanticipins present in the plants. On attack of microorganisms, these glycosides hydrolysed themselves to release aglycones which have inhibitory effects against the attacking microorganisms (Osbourn, 1996). On the other hand phytoalexins are produced in the plant against some stress which may be environmental, herbivorous or microbial. These have lower molecular mass and may include phenolic acids, flavonoids or phenylpropanoid derivatives (Grayer and Harborne, 1994).

Several reports have been published in the literature that ascertain the antimicrobial activity of phenolic compounds and the extracts rich in phenolics (Amelia *et al.*, 2006; Atrott and Henle, 2009; Chakraborty *et al.*, 2007; Ferrazzano *et al.*, 2009; Harrison *et al.*, 2003; Li and Xu, 2008; Pereira *et al.*, 2007). These reports indicate the phenolic compounds and the extracts rich in phenolic compounds as a strong alternative candidate to synthetic food preservatives and antibiotics (Oliveira *et al.*, 2007).

Cueva *et a.*, (2010) evaluated the antimicrobial activity of thirteen (13) phenolic acids including Benzoic acid, 3-Hydroxybenzoic acid, 4-Hydroxybenzoic acid, Protocatechuic acid, Vanillic acid, Phenylacetic acid, 3-Hydroxyphenylacetic acid, 4-Hydroxyphenylacetic acid, 3,4-Dihydroxyphenylacetic acid, Phenylpropionic acid, 3-Hydroxyphenylpropionic acid, 4-Hydroxyphenylpropionic acid and 3,4-Dihydroxyphenylpropionic acid against different strains of *E. coli*, *Lactobacillus* species., *S. aureus*, *P. aeruginosa* and *C. albicans*. They used three strains of *E.coli* including ATCC 25922, CECT 5947 and lpxC/tolC. The E.

coli lpxC/tolC was inhibited by all the phenolic acids tested for their inhibitory effect and the pathogenic strain CECT 5947 was susceptible to ten phenolic acids (Benzoic acid, 3Hydroxybenzoic acid, 4-Hydroxybenzoic acid, Vanillic acid, Phenylacetic acid, 3-
Hydroxyphenylacetic acid, 4-Hydroxyphenylacetic acid, Phenylpropionic acid, 3Hydroxyphenylpropionic acid and 4-Hydroxyphenylpropionic acid) out of thirteen phenolic acids applied while non-pathogenic strain ATCC 25922 of *E.coli* was inhibited by only four phenolic acids (Benzoic acid, Vanillic acid, Phenylacetic acid and Phenylpropionic acid). Inhibitory effect of phenolic acid was also determined against five species of *Lacto bacilli* and found that phenolic acids were the most effective against *L. paraplantarum* while least effective against *L. fermentum*. They also noted that *P. aeruginosa* was resistant to all the phenolic acids tested. Chakraborty *et al.*, (2007) reported that caffeic acid and rosemaric acid isolated from the leaves of *Basilicum polystachyon* were highly effective against gram positive bacteria and *A. niger* .

Effectiveness of flavonoids against microorganisms is widely accepted. In this regard, many reports have been published about the inhibitory effect of commercially available flavonoids and the flavonoids extracted/isolated from the natural sources. Flavonoids whose antimicrobial activity has been reported include apigenin (Basil *et al.,* 2000; Sato *et al.,*2000) catechin (Hirasawa and Takada, 2004; Kumbukgolla *et al.*, 2007; Kuete *et al.,* 2008), naringin and naringenin (Ng *et al.,*1996; Rauha *et al.,*2000; Mandalari *et al.*, 2007) epigallocatechin gallate and its derivatives (Yee and Koo 2000; Zhao *et* al., 2001; Stapleton *et* al., 2004; Taguri *et al.,*2004), luteolin and luteolin 7-glucoside (Suksamrarn e*t al.*, 2004; Anabela *et al.*, 2006; Kuete *et al.,* 2008; Salawu *et al.,*2011), myricetin (Demetzos *et al.*,
2001) quercetin, 3-*O*-methylquercetin and various quercetin glycosides (Rauha *et al.,*2000; Arima and Danno 2002; Li and Xu, 2008) and rutin (Salawu *et* al.,2011) kaempferol and its derivatives (Rauha *et al.,*2000).

Salawu *et al.*, (2011) identified phenolic compounds in *V. amygdalina, O.*

gratissimum and *M. utilissima* and then evaluated the antimicrobial activity of main phenolic compounds (rutin, luteolin, luteolin 7-glucoside, caffeic acid and Rosmarinic acid) which were identified in the botanical samples against *S. aereus, B. cereus* and *Shigella spp* at different concentrations (10, 25, 50 mg/mL). They concluded that inhibitory effect of these compounds was concentration dependent. Li and Xu, (2008) also reported the antimicrobial effect of quercetin isolated from the leaves of lotus.

Kuete *et al*., (2008) isolated different flavonoids (Alpinumisoflavone, Catechin, Epiafzelechin , Genistein , Laburnetin and Luteolin) from the extracts of two *Ficus* species (*Ficus chlamydocarpa*, *Ficus cordata*) and studied the antimicrobial activity of crude extract and isolated flavonoids against fungi and bacteria. They reported that Luteolin and Epiafzelechin exhibited their inhibitory effect against all the microbial strains used. He also reported that Laburnetin, Alpinumisoflavone and Genistein were active against fourteen, eight and seven microbial strains respectively out of sixteen strains applied to evaluate their activity.

2.7.2. Antimicrobial activity of selected plants of *Ficus* species

Plants remain under the focus of researchers for the discovery of novel and natural antimicrobial agents due to resistance developed by the microorganisms against the synthetic antimicrobial drugs (Turkoglu, *et al*., 2007; Hemaiswarya *et al.*, 2008; Alviano and Alviano, 2009). On the other hand growing concerns of the consumers against the synthetic food preservatives also stimulate the research to explore the antimicrobial potentials of plants (Cock, 2008; Khan *et al*., 2009). Remedial potential of certain plants against infectious diseases was accepted even before the discovery of microorganisms (Anwar *et al*., 2009). Antimicrobial activity of plants from all over the world has been investigated and reported by different researchers (Harrison *et al*., 2003; Pereira *et al*., 2007; Tomori *et al*., 2007; Ao *et* al., 2008; Arwa *et al*., 2008; Ferrazzano *et al*., 2009; Hamid *et al*., 2009; Alimuddin *et al*.,2010).

Plants belonging to the genus *Ficus* are also investigated for their antimicrobial activity as they have been used in folk medicinal systems for the treatment of infectious diseases. Ao *et al*., (2008) evaluated the antimicrobial potential of methanolic extracts from different parts (bark, fruit and leaves) of *F. microcarpa L* against *Bacillus brevis, Bacillus cereus, Bacillus subtilis, Escherichia coli and Achromobacter polymorph*. They concluded that ethyl acetate fraction of the crude extract from the bark has highest antimicrobial potential as compared to the other samples investigated. Adebayo *et al*., (2009) reported that bark, leaf and root of *F. exasperate* has significant inhibitory effect against human pathogenic bacteria. *B. subtilis* and *P. aeruginosa* were found remarkably sensitive to the aqueous extract of *F.religiosa* (Preethi *et al*., 2010). In another study Aref *et al*., (2010) used chloroform, ethyl acetate, hexane and methanol for extraction from the latex of *F. carica* and evaluated these extracts for their antimicrobial activity and reported that all the extracts exhibited remarkable inhibition against the tested strains. *F. auriculata* which is widely used for the treatment of cholera, diarrhoea, dysentery, wounds etc. t has antimicrobial activity (Gairola and Biswas, 2008). On this basis Gair *et al*., (2011) studied the antimicrobial activity of the extract of *F. auriculata* stem bark in chloroform, hexane and methanol against *Escherichia coli* and *Staphylococcus aureus*. Alaribe *et al*., (2011) conducted a study to estimate the antimicrobial activities of hexane extract of stem bark of *F. congensis* against *E. coli, B. substilis, K. pneumonia, S. aureus, A. fumigatus, T. mentagrophytes, T. rubrum* and *C. albicans*. He concluded that the hexane extract was most active against *E. coli* among the tested bacterial strains while it was effective only against *C. albicans* among the tested fungi. Shabir *et al*., (2011) studied the antioxidant and antimicrobial properties of the *Delonix regia* by using absolute ethanol, methanol, aceton, 80% aqueous ethanol, 80% aqueous methanol and 80% aceton as solvent for extraction. They concluded that the solvent (80% methanol) exhibiting higher antioxidant activities also exhibited higher antimicrobial activities.

2.7.3. Methods for evaluation of Antimicrobial activity

Agar diffusion and agar/broth dilution tests are widely used for evaluation of antimicrobial activity of plant extracts. These two commonly used methods are also known as "screening methods" and used to determine the potential usefulness of the extracts against different microbes.

Agar diffusion test is generally first step to explore the antimicrobial potential of plant extracts. There are many variations of this assay but the most common variations are disk diffusion and well diffusion. An antimicrobial agent or plant extract is applied to the surface of an agar medium that has been inoculated with the test organism, using an impregnated filter paper disk or placed in a well. Antimicrobial compound present in the disk or well diffuses through the agar and a concentration gradient is developed. As the distance from the disc increases, the concentration of the test substance decreases logarithmically. In areas where the concentration of extract is inhibitory, no growth occurs; forming a zone of inhibition around the disc and the inhibition is represented by the diameter of this zone. The results obtained by this method are generally qualitative and microorganisms tested against a specific extract are termed as susceptible, intermediate or resistant on the basis of diameter of zone.

The strength of antimicrobial activity is determined by agar/broth dilution test. In this method, a compound or extract is diluted serially and distributed in agar or in a nutrient broth, which then is inoculated with a single strain of microorganism. The broth dilution assay can be used in both macro and micro-dilution versions. The use of micro-tube version increases the productivity through the use of micro-titre plates. The objective of both the agar and broth dilution assays is to describe the inhibition of a microorganism at a specific end point in specific time duration. The measurement of inhibition at a specific time is termed as minimum inhibitory concentration (MIC). The MIC can be defined as the lowest concentration at which no growth occurs in a nutrient medium. Another variation of microdilution method is the use of redox indicator resazurine as a visual indicator of the MIC and this method is more sensitive than agar dilution method (Mann and Markham, 1998).

Chapter-3

MATERIALS AND METHODS

The work presented in this thesis was carried out in different well reputed institutions. The experimental work regarding antioxidant activities of the samples was performed in the Analytical Laboratory, Department of Chemistry, University of Agriculture, Faisalabad, Pakistan. HPLC analysis for phenolic compounds and antimicrobial activities of the samples were carried out in the Department of Food Science and Technology, University of Georgia, Athens, GA, USA. The Scanning Electron Microscopic (SEM) images of the samples were taken at Centre for Advanced Ultrastructural Research (CAUR), University of Georgia, Athens GA, USA.

3.1. Collection of samples

Five species of genus *Ficus* (*F. bengalensis, F. infectoria, F. racemosa, F. religiosa, F. retusa*) were selected on the basis of their ethno-medicinal usage in local medicinal system and wide distribution/occurrence in Pakistan. Fruits, leaves and barks of the selected species were harvested/collected in the third week of April, 2010 in the vicinity of University of Agriculture, and Forest Research Park Gatwala, Faisalabad. The specimen were further identified and authenticated by taxonomist Dr. Mansoor Ahmed, associate professor Department of Botany, University of Agriculture, Faisalabad, Pakistan. The collected samples were cleaned, dried in oven at 45 °C until constant mass achieved. The dried samples were ground by using a grinder (TSK-949, Westpoint, France) and then stored in a refrigerator at 4 °C for investigation.

3.2. Description of the analytical instruments used throughout the research work

3.2.1. Hot air oven (IM-30, Irmeco, Germany)

3.2.2. Grinder (TSK-949, Westpoint, France)

3.2.3. Orbital shaker (Gallenkamp, UK)

3.2.4. Rotary vacuum evaporator (EYELA, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan)

3.2.5. Spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan)

3.2.6. HPLC (Thermo Finnigan (TSP00-0301))

3.2.7. Scanning Electron Microscope (Zeiss 1450 EP)

3.2.8. Conductivity meter

3.2.9. Biological Safety Cabinets (Nuair NU-425-600)

3.2.10. Autoclave (Primus Sterlizer Co. Inc)

3.2.11. Automatic Plate Dispenser (Brunswick Scientific Co. Inc: MP-1000)

3.3. Reagents and standards

Chemicals and reagents used during this research work were of analytical grade. HPLC grade solvents were used as mobile phase for the analysis of phenolic compounds. All the chemicals and reagents used were of Merck, Sigma or Fluka brand. 1,1,-diphenyl-2picrylhydrazyl radical (DPPH) (Sigma, 90.0 %), linoleic acid, food grade synthetic antioxidant butylated hydroxytoluene (BHT) (99.0 %), Folin-Ciocalteu reagent (2 N) and standards of phenolic acids (Gallic acid, Protocatechuic acid, 2,5-dihydroxy benzoic acid, Chlorogenic acid, Vanillic acid, Caffeic acid, Syringic acid, p-Coumaric acid, Ferulic acid and Sinapic acid) and flavonoids (Rutin, kampeferol, quercetin, luteolin and myricetin) were purchased from Sigma Chemicals Co (St, Louis, MO, USA). All other chemicals (analytical grade) i.e. acetic acid, anhydrous sodium carbonate, sodium hydroxide, sodium nitrite, ammonium thiocyanate, ferrous chloride, potassium dihydrogen phosphate, aluminum chloride, dipotassium hydrogen phosphate, potassium iodide, and sodium thiosulphate used in this study were purchased from local distributer of Merck (Darmstadt, Germany) in Pakistan, unless stated otherwise. The reagents and chemicals used for antimicrobial activity were purchased from Oxoid including Nutrient Agar, Nutrient Broth, Sabouraud Desxtrose Broth, Potato Dextrose Agar, Resazurin, Amoxycilline (AML), Fluconazole (FCA), Flumequine (UB), Amoxycilline (AML) and Ampicillin (AMP).

3.4. Experimental protocol

3.4.1. Effect of extraction medium/technique on the antioxidant activity of plant materials

3.4.1.1. Extracting solvent systems

The samples were ground into a fine powder using a commercial blender (TSK-949, West point, France). The ground material was passed through 80-mesh sieve and then it was used for extraction purposes. Four different solvent systems i.e. 100% ethanol, 100% methanol, 80% ethanol (ethanol: water, 80:20 v/v), and 80% methanol (methanol: water, 80:20 v/v) were employed in order to evaluate the effects of extraction media on yield and antioxidant activity in extractable components of the plant.

3.4.1.2. Extraction techniques

To evaluate the effect of extraction technique on the extraction yield and other antioxidant properties of the plant extracts, three different techniques (Orbital shaker, Magnetic stirring and Ultrasound extraction) were applied using all the solvents mentioned in 3.4.1.1 separately in each technique.

3.4.1.2a. Extraction by orbital shaker

Twenty grams of each ground sample was extracted by using 200 mL of each solvent separately for 8 hours at 35 ^{0}C in a temperature controlled orbital shaker (Gallenkamp, UK). Then this mixture of solvent and sample was filtered using filter paper (Whatman No. 1). In this way the residue obtained was extracted twice with the same fresh solvent in the same manner as mentioned above. All the three filtrates were combined and concentrated using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan) under reduced pressure at 45 ^{0}C. These concentrated and dried extracts were weighed for the calculation of % age yield and stored at - 4

^{0}C in a refrigerator, until used for further analyses. Solvent extraction of each ground sample was done in triplicate by using the orbital shaker as extraction technique.

3.4.1.2b. Extraction by using magnetic stirring

Twenty grams of each ground sample was extracted by using 200 mL of each solvent separately for 8 hours at 35 ^{0}C by a temperature controlled magnetic stirring. Then this mixture of solvent and sample was filtered using filter paper (Whatman No. 1). In this way the residue obtained was extracted twice with the same fresh solvent in the same manner as mentioned above. All the three filtrates were combined and concentrated using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan) under reduced pressure at 45 °C. These concentrated and dried extracts were weighed for the calculation of % age yield and stored at - 4 ^{0}C in a refrigerator, until used for further analyses. Solvent extraction of each ground sample was done in triplicate by using the magnetic stirring as extraction technique.

3.4.1.2c. Ultrasound assisted extraction

Twenty grams of each ground sample was extracted by using 200 mL of each solvent separately for one hour and 30 minutes at 35 ^{0}C in a temperature controlled ultrasound bath. Then this mixture of solvent and sample was filtered using filter paper (Whatman No. 1). In this way the residue obtained was extracted twice with the same fresh solvent in the same manner as mentioned above. All the three filtrates were combined and concentrated using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan) under reduced pressure at 45 °C. These concentrated and dried extracts were weighed for the calculation of % age yield and stored at - 4 ^{0}C in a refrigerator, until used for further analyses. The above mentioned procedure was repeated in triplicate for each sample to get three extracts of each sample.

3.5. Evaluation of antioxidant activity of plant materials/extracts

Different *in-vitro* antioxidant assays were used in the present study to evaluate the antioxidant activity of the extracts. These methods were slightly modified where required according to the nature of samples. Brief description of the tests performed is given below:

3.5.1. Determination of total phenolics content (TPC)

Total phenolics content of the samples were assessed by using the method described by Singleton *et al.*, (1965) and later followed by many researchers (Anwar *et al.*, 2009, Sultana *et al.*,2008, Zubair *et al.*, 2012). In this method, 2 mL of the sample solution containing 1mg/mL of extract was mixed with 1mL of Folin-Ciocalteu reagent and 5mL of deionized water. After 10 minutes, 10% Na_2CO_3 was added and this mixture was heated at 40 ^{0}C for 10 minutes on a water bath and then the temperature of the mixture was brought to room temperature. Similarly gallic acid solutions of different concentrations (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1mg/mL) were prepared. The absorbance of all the samples and standard solutions was measured at 755 nm with the help of spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Total phenolic contents of each extract were calculated in triplicate with the help of gallic acid calibration curve and the results were reported as gallic acid equivalents in grams per 100 gram of dry sample (GAE g/100g DW).

3.5.2. Determination of total flavonoid contents (TFC)

The TFC of the extracts from the samples were determined by using spectrophotometric method as described previously by Sultana *et al.* (2008) with slight modifications according to the requirements. One mL solution of plant extract of each sample having concentration 1 mg/mL was diluted with 4 mL water in a volumetric flask of 10 mL capacity. Then, 0.3 mL of $NaNO_2$ (5% solution) was mixed in each volumetric flask; after 5 minutes, 0.3 mL of $AlCl_3$ 10% (w/v) solution was added; then after 6 minutes, 2 mL of sodium hydroxide solution of (1 *M)* was added. Then volume of this mixture was made up to 10 mL by adding distilled water and mixed well. Absorbance of this mixture was noted at 510 nm wavelength with the help of spectrophotometer. The results were expressed as catechin equivalents gram per 100 gram of dry weight (CE g/100g DW). Three readings were taken for each sample and the results were averaged.

3.5.3. DPPH. Scavenging assay

Free radical scavenging activity of the samples was determined by using 1, 1'– diphenyl–2-picrylhydrazyl (DPPH) method as reported earlier (Anwar *et al.*,2009). Different concentrations (0.02, 0.04, 0.06, 0.08, 0.1mg/mL) of extract from each sample were prepared in methanol. Then 50 µL of each extract solution was added to 5mL of freshly prepared 0.1mM DPPH solution separately in test tubes. These test tubes were placed at room temperature in darkness. After 30 minutes, the absorbance of each reaction mixture/solution was measured at wavelength of 517 nm with the help of spectrophotometer. Then % age inhibition of each sample was calculated by using formula:

$$I(\%) = 100 \times (A_{blank} – A_{sample}/A_{blank})$$

Where A_{sample} was the absorbance of DPPH solution containing solution of the extract of samples and A_{blank} was the absorbance of DPPH solution only. IC_{50} (Extract concentration providing 50% inhibition) for each sample was calculated by plotting graph of %age inhibitions against different concentrations of same sample.

3.5.4. Determination of antioxidant activity in linoleic acid system

The antioxidant activity of the sample extracts was also assessed by measuring the percent inhibition of linoleic acid oxidation (Iqbal *et al.*, 2005). Each plant extract (5 mg) was added separately to a solution constituting 0.13 mL of linoleic acid, 10 mL of absolute ethanol and 10 mL of 0.2 M sodium phosphate buffer (pH 7). The mixture was made up to 25 mL with distilled water and incubated at 40 ^{o}C for 360 hours. Extent of oxidation was measured by measuring peroxide value applying thiocyanate method as described by Yen *et al.*, (2000). Briefly, 10 mL of ethanol (75% v/v), 0.2 mL of aqueous solution of ammonium thiocyanate (30% w/v), 0.2 mL of sample solution and 0.2 mL of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5% HCl; v/v) added sequentially. After 3 min of stirring, the absorption was measured at 500 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). A control contained all reagents with exception of extracts. Synthetic antioxidants butylated hydroxytoluene (BHT) was used as positive control. Percent of inhibition of linoleic acid oxidation were calculated with the following equation: 100 – [(Abs. increase of sample at 360 h / Abs. increase of control at 360 h) / 100], to express antioxidant activity.

3.5.5. Determination of reducing power

To determine the reducing power of the samples under investigation the method described by Yen *et al.*, (2000), was used with slight modification. Different concentrations (0.2-1mg/mL) of the extracts was prepared and mixed with 5 mL of 0.2 *M* sodium phosphate buffer having 6.6 pH and 5 mL of 1% potassium ferricyanide. The mixtures were incubated for 25 minutes at 40 °C. Then the mixtures were cooled to room temperature followed by addition of trichloroacetic acid (5.0 mL, 10.0%), mixing and centrifugation at 5 °C for 10 minutes. Then 5 mL of the upper layer of the reaction mixture was taken and diluted with equal amount of distilled water. After that 1 mL of 0.1% $FeCl_3$ was added to this diluted mixture, the absorbance of the final mixture was noted at wavelength of 700 nm using spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan).

3.6. Identification and quantification of phenolic acids and flavonoids

The most potent antioxidant extracts obtained from the plant samples by using 80% methanol as solvent and sonication as extraction procedure were analysed by employing Thermo Finnigan (TSP) HPLC for the identification and quantification of selected individual phenolic acids and flavonoids. The HPLC system was equipped with TSP-P4000 pumping system, TSP-AS3000 autosampler, SUPELCOSIL™ LC-18 HPLC Column (5 µm particle size, L × I.D. 25 cm × 4.6 mm) and TSP-UV3000 detector. Prior to HPLC analysis, extract was hydrolysed following the method described by Tokusoglu *et al.* (2003) to convert bound form phenolic acids and flavonoids into free form aglycons . Briefly acidified methanol (25 mL) containing 1 % (v/v) HCl and 0.5 mg /mL BHT was added to each plant extract (5 g). HCl (1.2 *M*, 5 mL) was also added and the mixture was stirred at 90 °C under reflux for 2 hours. The reaction mixture was cooled to room temperature and centrifuged at 5000 rpm for 10 min. Upper layer of the reaction mixture was taken and sonicated for 5 minute to remove any air present. The final extracts were filtered through a 0.45-µm (Millipore) before injecting into HPLC. Phenolic acids and flavonoids were separated and quantified using HPLC conditions represented in table 3.1.

Phenolic compounds were identified and quantified by using external standard method, for this purpose standard stock solution of ten authentic phenolic acids standards including caffeic acid, chlorogenic acid, p-Coumaric acid, ferulic acid, gallic acid, gentisic acid, protocatechuic acid, sinapic acid, syringic acid and vanillic acid were prepared by dissolving 1mg in 10ml methanol and further diluted to 0.08, 0.11, 0.14, 0.17, 0.2 mg/Litre for standard curve preparation. Precision was determined by analyzing 10 µl of each standard solution. (n=3). The peak areas were automatically measured by an integrator of HPLC instrument.

Calibration curve for each standard was obtained by analysing five different concentrations (0.08, 0.11, 0.14, 0.17, 0.2 mg/Litre) of each sample and then by plotting the peak area against concentration. This showed linearity in accordance to Beer's law. The limit of detection under the present chromatographic conditions were evaluated at S/N of 3. Flavonoids were identified from the samples chromatogram in the similar manner by using the conditions mentioned in tables 3.1 for flavonoids. Five authentic flavonoids standards including kaempferol, luteolin, myricetin, quercetin and rutin were used in this study for their identification in the samples.

Table 3.1. HPLC conditions for the analysis of phenolic compounds

	Phenolic compounds	
	Phenolic acids	Flavonoids
Column	SUPELCOSIL™ LC-18 (5 µm particle size, L × I.D. 25 cm × 4.6 mm)	SUPELCOSIL™ LC-18 (5 µm particle size, L × I.D. 25 cm × 4.6 mm)
Mobile phase	[A: H2O containing 0.02% Triflouroaceticacid, B: MeOH containing 0.02% triflouroacetic acid (v/v)] (isocratic elution), flow rate: 1 mL /min	[A: 3% aq. Triflouroacetic acid, B: Acetonitrile: MeOH (80: 20 v/v)] (isocratic elution), Flow rate: 1.0 mL /min

Detector	UV (280 nm)	UV (360 nm)

3.7. Evaluation of Antimicrobial Activities

The most potent antioxidant extracts obtained by using 80% methanol and sonication extractions were individually tested against a panel of microorganisms selected. Bacterial strains were cultured overnight at 37 ºC in nutrient agar (NA) while the fungal strains cultured overnight at 30 ºC using potato dextrose agar (PDA). Different growth media were prepared by following the instructions given by the company. Following antimicrobial assays were employed for the determination of antimicrobial potential of the samples.

3.7.1. Disc Diffusion Method

This test was performed by using the approved performance standards given in M2A9, volume 26 No.1 (2006) by Clinical and Laboratory Standards Institute (CLSI). The dried extracts of the samples were dissolved in DMSO to a final concentration of 10 mg/mL and sterilized by filtering it through 0.45 µm Millipore filters. Then sterilized 6 mm disks in diameter were impregnated with above mentioned 50 µl of extract solution in DMSO. Prior to this, equal amount (20 mL) of the growth medium was placed on sterilized flat bottom petri dishes with the help of automatic plate dispenser and allowed to cool at room temperature. Then these petri dishes were inoculated with 500 µL of suspension of microbes (bacteria or fungi) having turbidity equivalent to a 0.5 McFarland standard. Then disks impregnated with samples and control was placed on the inoculated growth media in petri dishes and these were incubated at 35 ^{0}C for 16 to 18 hours. Finally zones of inhibition were measured to evaluate the antimicrobial activity.

3.7.2. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of only those samples was determined which were found sensitive to the microbes in the disc diffusion test. Minimum inhibitory concentration was determined by modified resazurin microtitre-plate as reported by Sarker *et al.*, (2007). Briefly, 100 µL of sample solution having concentration of 10mg/mL and standard antibiotic (1 mg/mL in 10% DMSO) was pipetted into the first row of the 96 well plates. To all other wells 50 µL of nutrient broth was added. Two fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. 30 µL of 3.3x strength isosensitised broth and 10 µL of resazurin indicator solution (prepared by dissolving 270 mg tablet in 40 mL of sterile distilled water) were added in each well. Finally, 10 µL of bacterial suspension was added to each well to achieve a concentration of approximately 10^8 cfu mL^{-1}. Each plate had a set of controls: a column with a ciprofloxacin as positive control, a column with all solutions with the exception of the test compound, a column with all solutions with the exception of the bacterial solution adding 10 µl of nutrient broth instead and a column with solvent (v/v) solution as a negative control. The plates were prepared in triplicate.

Plates were enfolded loosely with cling film and incubated at 37 °C for 24 h. The colour change was then assessed visually. The growth was indicated by colour changes from purple to pink or colourless. The lowest concentration at which colour change occurred was taken as the MIC value.

3.8. Evaluation of antiscalant activity

3.8.1. Brine solution preparation

First of all $CaCl_2$ brine solution, having ionic strength similar to sea water, was prepared by the proportions described by Abdel-Gaber *et al.*, 2008, dissolving 40.95g/lit of NaCl (0.7 M), 0.21 g/litter of $NaHCO_3$ (0.0025M), 4 g/litter of Na_2SO_4 (0.028M) and 0.95 g/litter of $CaCl_2$ (Lin and Dexter, 1988; Abdel-Gaber *et al.*, 2008).

3.8.2. Conductivity test

All the apparatus (glass beakers, stirrer and conductivity sensor) used in the test set-up was thoroughly washed with sulphuric acid (1M) and distilled water so that any deposits which can be a source of crystal nuclei can be removed. Different concentrations (25 ppm, 50 ppm, 75 ppm and 100 ppm) of the sample extracts were prepared by taking calculated amount of the extract, adding 5ml of

$CaCl_2$ brine solution and making the volume 100ml by using deionized water. Then 10ml of all the sample solutions and blank (containing only $CaCl_2$ brine solution) were titrated against 0.1 M Na_2CO_3 separately.

3.8.3. Scanning Electron Microscopic (SEM) Examination

Steel strips of 1cm width and 8cm length were taken and covered with resin in such a way that only $1cm^2$ areas from one side was left uncovered. This uncovered area was abraded mechanically with emery papers. Then these strips were used as cathode and anode by hanging two strips in 50 ml of $CaCl_2$ brine solution and by connecting one strip to positive terminal and other with negative terminal of a battery of 1.2 volts. The current was allowed to pass through the solution for twenty hours. Then scale formed on the surface of cathode was examined by SEM (Zeiss 1450 EP). This experiment was repeated separately by using $CaCl_2$ brine solution containing 25 ppm, 50 ppm and 100 ppm of the extracts of each sample. Then SEM images of deposition of scale on the cathode in the absence and in the presence of different concentration of the samples extract were taken and compared to evaluate the effect of the sample extract on the scale formation.

3.9. Statistical analysis

Three samples of each plant material were assayed. Each sample was analysed individually in triplicate for their chemical composition, antioxidant and antimicrobial potential. The data has been reported as mean (n = 1 x 3 x 3) ± standard deviation and analysed by analysis of variance (ANOVA) using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennysalvania, USA) at 5% significance level.

Chapter-4

Results and Discussion

In this chapter data obtained, after investigating the selected samples for their antioxidant, antimicrobial and antiscalant activities through various protocols has been described in chapter-3, is represented in the form of tables after statistical analysis. All the samples were analysed in triplicate and the mean of the three results along with standard deviation (mean±SD) is presented in tables. Different significant levels determined after performing analysis of variance (ANOVA) are mentioned with the help of different letters in subscripts and superscripts.

4.1. Influence of extraction process on extraction yield

The extraction yield of antioxidant/biologically active components from different parts of the selected species of *Ficus* was accomplished by using four solvents: 100% ethanol, 100% methanol, 80% ethanol (ethanol:water 80:20 v/v) and 80% methanol (methanol:water 80:20 v/v) and three extraction techniques i.e. orbital shaker, sonication, magnetic stirring. The results of extraction yield from fruits, leaves and barks of the selected *Ficus* species are summarized in the table 4.1, 4.2 and 4.3 respectively.

In the present study 80% methanol proved to be the best solvent for extraction of antioxidant compounds from different fruit and leaf samples of the selected species of *Ficus*. In case of bark samples investigated in the present study 80% ethanol offered the significantly higher yields in comparison to other solvents applied for extraction. Sultana *et al.*, (2009) conducted a study to evaluate the effect of extraction procedure on the extraction yield from leaves and roots of *Moringa oleifera*, leaves of *Aloe barbadensis*, fruits of *Ficus religiosa*, and barks of *Azadirachta indica*, *Acacia nilotica*, *Eugenia jambolana*, *Terminalia arjuna* by applying the same solvents used in present study. In agreement with the results of present study they found that 80% methanol was the most efficient solvent for extraction of antioxidant compounds from the fruits, leaves and roots while 80% ethanol extracted maximum amount of antioxidants from the bark samples. In fact, the polarity of solvent has great effect on the solubility of different components present in the plant and many researchers concluded that higher yields could be achieved by using more polar solvent (Anwar *et al.*, 2003; Sidhuraju *et al.*, 2003).

As far as, the effect of extraction techniques on the yield of antioxidant components is concerned, sonication based extraction proved to be the best technique in comparison with the magnetic stirring and orbital shaking. Vigour of shaking and force applied on the sample's particles also affect the extraction yield (Siddhuraju and Becke, 2003). In sonication, high frequency sound waves (>20 kHz) called ultrasound are passed through the solvent containing sample solid particles. When these wave strikes the solid particles a parallel or perpendicular force is generated and these forces as a result generate shear waves (in case of parallel force) and compressive waves (in case of perpendicular force). During the formation of these waves bubbles grow and collapse. Collapse of the bubbles produces shock waves and sonic energy is transformed in to mechanical energy which is equivalent to several thousand of atmospheric pressure (Junior *et al.*, 2006). This increase in pressure disrupts the cellular membrane and facilitates the movement of solvent in to cell and the desired components are migrated to solvent. Many researchers applied this technique on different plant material like soybeans (Rostagno *et al.*, 2003), wheat bran (Wang *et al.*, 2008) and coconut shell powder (Rodrigues and Pinto, 2007) and found that sonication was an effective tool for the extraction of bioactive components from plant materials. In the present study we got the highest yield with sonication assisted extraction provided that the solvent composition was maintained the same.

Yields of extractable components from the fruit samples varied significantly ($p \leq 0.05$) under the influence of different solvent systems applied for extraction. Data in the table 4.1 revealed that significantly ($p \leq 0.05$) higher yields were obtained when 80% methanol was used as solvent. Significantly higher yields ($p \leq 0.05$) were obtained with sonication assisted extraction as compared to other two techniques (orbital shaking and magnetic stirring) applied for extraction. Combination of sonication assisted extraction technique and 80% methanol as extraction solvent provided significantly higher ($p \leq 0.05$) yields in comparison to all other combinations of extraction technique and solvent for the fruit of same specie. For the fruits of *F.bengalensis* the yields of extractable components ranged from 10.73±0.49 to 23.88±1.12 and the ranges for the extraction yields of fruit samples of *F. religiosa*, *F. retusa* and *F.infectoria* were from 14.81±0.63 g/100 g DW to 30.5±1.2 g/100 g DW, from 12.47±0.51 g/100 g DW to 25.4±1.05 g/100 g DW and from 11.64±0.52 g/100 g DW to 21.07±0.96 g/100 g DW, respectively. The highest yield for each fruit sample was significantly (p≤0.05) higher from the lowest ones for each fruit sample and obtained by the combination of 80% methanol with sonication while the

Table 4.1. Effect of extraction procedure on the %age yield of extract obtained from the fruits of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
F. bengalensis	Orbital Shaker	$10.73\pm0.49_{c}^{c}$	$15.33\pm0.71_{c}^{b}$	$11.29\pm0.52_{c}^{c}$	$22.37\pm0.99_{c}^{a}$
	Sonication	$12.84\pm0.60_{a}^{c}$	$17.23\pm0.80_{a}^{b}$	$12.46\pm0.59_{a}^{c}$	$23.88\pm1.12_{a}^{a}$
	Magnetic Stirrer	$11.17\pm0.52_{b}^{c}$	$15.84\pm0.74_{b}^{b}$	$11.92\pm0.56_{b}^{c}$	$22.68\pm0.99_{b}^{a}$
F. infectoria	Orbital Shaker	$11.64\pm0.52_{b}^{d}$	$16.88\pm0.74_{b}^{b}$	$13.08\pm0.61_{b}^{c}$	$18.6\pm0.86_{b}^{a}$
	Sonication	$13.27\pm0.62_{a}^{d}$	$19.88\pm0.91_{a}^{b}$	$14.97\pm68_{a}^{c}$	$21.07\pm0.96_{a}^{a}$
	Magnetic Stirrer	$11.9\pm0.56_{b}^{d}$	$17.6\pm0.79_{b}^{b}$	$12.77\pm0.60_{b}^{c}$	$19.3\pm0.91_{b}^{a}$
F. Racemosa	Orbital Shaker	$12.15\pm0.55_{ab}^{c}$	$18.32\pm0.79_{ab}^{b}$	$13.93\pm0.64_{ab}^{c}$	$25.61\pm0.95_{ab}^{a}$
	Sonication	$14.81\pm0.65_{a}^{c}$	$19.97\pm0.91_{a}^{b}$	$15.24\pm0.72_{a}^{c}$	$26.71\pm1.11_{a}^{a}$
	Magnetic Stirrer	$9.37\pm0.43_{b}^{c}$	$18.45\pm0.84_{b}^{b}$	$13.10\pm0.62_{b}^{c}$	$25.94\pm1.20_{b}^{a}$
F. Religiosa	Orbital Shaker	$14.81\pm0.63_{b}^{c}$	$22.92\pm0.99_{b}^{b}$	$15.74\pm0.73_{b}^{c}$	$29.81\pm1.10_{b}^{a}$
	Sonication	$16.96\pm0.78_{a}^{c}$	$24.88\pm1.12_{a}^{b}$	$16.02\pm0.71_{a}^{c}$	$30.5\pm1.2_{a}^{a}$

	Magnetic Stirrer	$15.27\pm0.7_{b}^{c}$	$23.15\pm0.96_{b}^{b}$	$13.99\pm0.66_{b}^{c}$	$30.07\pm1.21_{b}^{a}$
F. retusa	Orbital Shaker	$12.47\pm0.51_{b}^{c}$	$16.28\pm0.74_{b}^{b}$	$12.82\pm0.59_{b}^{c}$	$23.77\pm0.92_{b}^{a}$
	Sonication	$15.49\pm0.70_{a}^{c}$	$18.17\pm0.84_{a}^{b}$	$14.25\pm0.66_{a}^{c}$	$25.4\pm1.05_{a}^{a}$
	Magnetic Stirrer	$12.87\pm0.58_{b}^{c}$	$16.74\pm0.72_{b}^{b}$	$13.42\pm0.63_{b}^{c}$	$24.18\pm1.02_{b}^{a}$

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

lowest yield for each fruit sample was obtained by the application of 100% ethanol using orbital shaker. For the fruits of *F. bengalensis*, the yields obtained by the application of three different techniques were significantly ($p \leq 0.05$) different. The yields obtained for the fruit samples of *F. infectoria, F. religiosa and F. retusa* by sonication were significantly ($p \leq 0.05$) different from the yields obtained by orbital shaking and magnetic stirring but there was no significant ($p \leq 0.05$) difference between the yields of these fruit samples obtained by orbital shaking and magnetic stirring. The extraction yields obtained with 80% methanol and 80% ethanol from all the fruit samples were significantly ($p \leq 0.05$) different from each other and from the yields obtained by the application of absolute methanol and absolute ethanol. The yields obtained by the application of absolute methanol and absolute ethanol from the fruits of *F.bengalensis, F. racemosa, F. religiosa* and *F. retusa* have no significant ($p \leq 0.05$) difference from each other but the results for the fruits of *F. infectoria* were significantly ($p \leq 0.05$) different.

The leaf samples of *Ficus* species investigated in the present study offered maximum yield when the extraction solvent was 80% methanol with sonication technique. The results obtained revealed that both the solvent and extraction technique has an appreciable effect on the extraction yield of the leaf samples. Table 4.2 represents the results for the leaf samples of selected species of *Ficus*. Yields of extractable components from the leaf samples improved significantly ($p \leq 0.05$) when different combinations of solvent with technique were applied for extraction. Extraction yield obtained from the leaf samples of *F. racemosa* increased by 45.9% when different combination of solvents with techniques were applied and it improved from 10.9±0.51 g/100 g DW to 20.14±0.92 g/100 g DW. Extraction yield from the leaves of *F. religiosa* increased from 10.52±0.47 to 19.07±0.88 and this enhancement was equal to 44.8%. The increase in the extraction yield from the leaf samples of *F. infectoria, F. bengalensi,* and *F. retusa* were 44%, (from11.65±0.58 to 20.84±0.95) 43.7% (from 10.24±0.42 to 18.21±0.81) and 41.6% (from 11.64±0.49 to 19.93±0.94), respectively. The yields obtained from the leaves of *F. bengalensis*, *F. racemosa* and *F. religiosa* by the application of different solvents were significantly ($p \leq 0.05$) different from one another and the statistical ranking of solvents for these plants was 80% methanol > 80% ethanol > 100% methanol > 100% ethanol. The statistical ranking of solvents on the basis of extraction yields obtained from the leaves of *F. infectoria* and *F. retusa* was 80% methanol > 80% ethanol > 100% methanol ≈ 100% ethanol. Sonication assisted extraction in comparison with other two techniques (orbital shaking and magnetic

Table 4.2. Effect of extraction procedure on the %age yield of extracts obtained from the leaves of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
F. bengalensis	Orbital Shaker	$10.24{\pm}0.42_c^d$	$15.80{\pm}0.71_c^b$	$10.98{\pm}0.47_c^c$	$16.34{\pm}0.77_c^a$
	Sonication	$11.35{\pm}0.53_a^d$	$16.93{\pm}0.79_a^b$	$12.14{\pm}0.57_a^c$	$18.21{\pm}0.81_a^a$
	Magnetic Stirrer	$10.95{\pm}0.51_b^d$	$16.26{\pm}0.74_b^b$	$11.35{\pm}0.53_b^c$	$16.64{\pm}0.78_b^a$
F. infectoria	Orbital Shaker	$11.65{\pm}0.58_b^c$	$17.72{\pm}0.83_b^b$	$11.56{\pm}0.54_b^c$	$19.34{\pm}0.9_b^a$
	Sonication	$15.89{\pm}0.75_a^c$	$19.65{\pm}0.92_a^b$	$14.45{\pm}0.67_a^c$	$20.84{\pm}0.95_a^a$
	Magnetic Stirrer	$12.9{\pm}0.58_b^c$	$18.14{\pm}0.85_b^b$	$11.66{\pm}0.55_b^c$	$19.37{\pm}0.88_b^a$
F. Racemosa	Orbital Shaker	$10.90{\pm}0.51_b^d$	$16.16{\pm}0.76_b^b$	$12.94{\pm}0.61_b^c$	$18.92{\pm}0.89_b^a$
	Sonication	$12.77{\pm}0.61_a^d$	$16.98{\pm}0.80_a^b$	$13.02{\pm}0.61_a^c$	$20.14{\pm}0.92_a^a$
	Magnetic Stirrer	$11.14{\pm}0.52_b^d$	$16.25{\pm}0.76_b^b$	$12.99{\pm}0.60_b^c$	$18.94{\pm}0.84_b^a$
F. Religiosa	Orbital Shaker	$10.52{\pm}0.47_b^d$	$14.86{\pm}0.69_b^b$	$11.56{\pm}0.49_b^c$	$16.64{\pm}0.78_b^a$
	Sonication	$12.21{\pm}0.54_a^d$	$16.90{\pm}0.73_a^b$	$13.84{\pm}0.65_a^c$	$19.07{\pm}0.88_a^a$
	Magnetic Stirrer	$10.6{\pm}0.5_b^d$	$15.05{\pm}0.7_b^b$	$11.82{\pm}0.55_b^c$	$16.70{\pm}0.78_b^a$
F. retusa	Orbital Shaker	$11.64{\pm}0.49_b^c$	$16.30{\pm}0.72\ _b^b$	$11.68{\pm}0.54_b^c$	$17.54{\pm}0.82_b^a$
	Sonication	$14.20{\pm}0.66_a^c$	$18.17{\pm}0.85_a^b$	$15.22{\pm}0.72_a^c$	$19.93{\pm}0.94_a^a$
	Magnetic Stirrer	$11.65{\pm}0.54_b^c$	$16.45{\pm}0.73_b^b$	$11.73{\pm}0.54_b^c$	$17.70{\pm}0.81_b^a$

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

stirring) provided significantly ($p \leq 0.05$) higher yields when applied for extraction. The yields obtained from the leaves of *F. bengalensis* by the application of orbital shaker, sonication and magnetic stirrer were significantly ($p \leq 0.05$) different and the ranking of extraction techniques was sonication > magnetic stirrer > orbital shaker. The ranking of extraction techniques on the basis of extraction yields obtained from the leaves of *F. infectoria*, *F. racemosa, F. religiosa* and *F. retusa* was sonication > magnetic stirrer ≈ orbital shaker. Combination of sonication assisted extraction technique and 80% methanol as extraction solvent provided significantly ($p \leq 0.05$) higher yields in comparison to all other combinations of extraction technique and solvent for the leaves of same specie. Comparison of the extraction yield obtained from the leaves of selected species of *Ficus* showed that the leaves of *F. infectoria* has the highest yield (20.84±0.95 g/100g of dry material) followed by leaves of *F. racemosa* (20.14±0.92 g/100g of dry material),leaves of *F. retusa* (19.93±0.94 g/100g of dry material), leaves of *F. religiosa* (19.07±0.88 g/100g of dry material) while the leaves of *F. bengalensis* gave the lowest yield (18.21±0.81 g/100g of dry material) among others, regardless of the extraction solvent or technique employed.

Extraction yields from the bark samples of selected species of *Ficus* were also improved significantly under the influence of different combinations of solvent with technique. The results revealed that 80% ethanol was the best solvent which offered the significantly ($p<0.05$) higher extraction yield from all the bark samples except from bark of *F.infectoria*. In case of *F.infectoria* the yields obtained from the bark by the application of 80% ethanol and 80% methanol were not significantly ($p<0.05$) different from each other. Sonication proved to be the most efficient extraction technique as was in case of fruit and leaf samples and the yields obtained by this technique were significantly ($p<0.05$) different from the yields obtained by the application of orbital shaker and magnetic stirring. Yields obtained by the application of orbital shaker and magnetic stirrer were not significantly ($p<0.05$) different from each other except for *F. bengalensis* where yields obtained by these extraction techniques were significantly ($p<0.05$) different. Although the polarity of solvent affects the extraction yield of the plant material but the chemical nature of extractable components in the plant tissues and their solubility in different solvents and the nature of matrix in which they are present decide the extraction yield of the samples. The extract yields were found to be varied significantly ($p<0.05$) in relation to different extraction solvents and the techniques. Typically, extraction yield of 7.28±0.35 g/100g DW from

Table 4.3. Effect of extraction procedure on the %age yield of extracts obtained from the bark of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$13.24\pm0.63_{c}^{c}$	$18.54\pm0.89_{c}^{a}$	$13.36\pm0.64_{c}^{c}$	$17.06\pm0.82_{c}^{b}$
	Sonication	$14.82\pm0.71_{a}^{c}$	$19.97\pm0.95_{a}^{a}$	$14.72\pm0.70_{a}^{c}$	$18.47\pm0.88_{a}^{b}$
	Magnetic Stirrer	$13.40\pm0.64_{b}^{c}$	$18.77\pm0.9_{b}^{a}$	$13.46\pm0.64_{b}^{c}$	$17.15\pm0.82_{b}^{b}$
F. infectoria	Orbital Shaker	$9.84\pm0.47_{b}^{b}$	$11.80\pm0.56_{b}^{a}$	$8.10\pm0.39_{b}^{c}$	$11.42\pm0.55_{b}^{a}$
	Sonication	$10.39\pm0.5_{a}^{b}$	$12.93\pm0.62_{a}^{a}$	$9.68\pm0.46_{a}^{c}$	$12.07\pm0.58_{a}^{a}$
	Magnetic Stirrer	$9.94\pm0.48_{b}^{b}$	$11.85\pm0.57_{b}^{a}$	$8.18\pm0.39_{b}^{c}$	$11.49\pm0.55_{b}^{a}$
F. Racemosa	Orbital Shaker	$7.28\pm0.35_{b}^{c}$	$10.93\pm0.52_{b}^{a}$	$7.02\pm0.34_{b}^{d}$	$10.01\pm0.48_{b}^{b}$
	Sonication	$8.74\pm0.42_{a}^{c}$	$11.9\pm0.57_{a}^{a}$	$8.25\pm0.39_{a}^{d}$	$11.50\pm0.55_{a}^{b}$
	Magnetic Stirrer	$7.39\pm0.35_{b}^{c}$	$11.06\pm0.53_{b}^{a}$	$7.11\pm0.34_{b}^{d}$	$10.43\pm0.5_{b}^{b}$
F. Religiosa	Orbital Shaker	$9.48\pm0.45_{b}^{c}$	$12.34\pm0.59_{b}^{a}$	$9.40\pm0.45_{b}^{c}$	$12.22\pm0.58_{b}^{b}$
	Sonication	$10.98\pm0.52_{a}^{c}$	$13.91\pm0.66_{a}^{a}$	$10.77\pm0.51_{a}^{c}$	$13.55\pm0.65_{a}^{b}$
	Magnetic Stirrer	$9.57\pm0.46_{b}^{c}$	$12.48\pm0.6_{b}^{a}$	$9.48\pm0.45_{b}^{c}$	$12.40\pm0.59_{b}^{b}$
F. retusa	Orbital Shaker	$11.30\pm0.54_{b}^{c}$	$16.76\pm0.8_{b}^{a}$	$11.58\pm0.55_{b}^{c}$	$15.70\pm0.75_{b}^{b}$
	Sonication	$12.17\pm0.58_{a}^{c}$	$18.22\pm0.87_{a}^{a}$	$14.18\pm0.68_{a}^{c}$	$17.07\pm0.82_{a}^{b}$
	Magnetic Stirrer	$11.43\pm0.55_{b}^{c}$	$16.91\pm0.81_{b}^{a}$	$11.68\pm0.56_{b}^{c}$	$15.87\pm0.76_{b}^{b}$

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

the bark of *F. racemosa* with least effective extraction system (absolute methanol and orbital shaker) increased to 11.90±0.57 g/100g DW with most effective extraction system (80% ethanol and sonication) which were significantly (p<0.05) different from each other. The yields obtained from the bark of *F. racemosa* by the application of different solvents used in the present study were significantly (p<0.05) different from each other. Increase in the yields, as function of least effective and most effective extraction system, from the barks of *F. retusa*, *F. infectoria*, *F. bengalensis* and *F. religiosa* was 38% (from 11.3±0.54 to18.22±0.87), 37.4% (from 8.1±0.39 to 12.93±0.62), 33.7% (from 13.24±0.63 to 19.97±0.95) and 32.4% (from 9.40±0.45 to13.91±0.66) respectively. Although the lower and higher values for above mentioned four species were significantly (p<0.05) different from each other but the yields obtained with absolute methanol and ethanol were not significantly (p<0.05) different from each other. Among the bark samples of the *Ficus* species scrutinized in the present study, *F. religiosa* offered the highest extraction yield while the bark of *F. racemosa* had the lowest yield regardless of the combination of solvents and techniques applied.

According to Ao *et al.*, (2008) the extract yield from fruit, leaves and bark of *F. microcarpa*, using 100% methanol as an extracting solvent, was found to be 11.8%, 14% and 15.5%, respectively. These reported values are in close agreement with our calculated extraction yields from fruit (14.25±0.66), leaves (15.22±0.72) and bark (14.18±0.68) of *F. retusa* (*F. microcarpa*) with methanol as solvent. The extraction yields, 15.74±0.73, 14.81±0.63, 29.81±1.1 and 22.92±0.99 g/ 100 g DW from fruit of *F. religiosa* obtained by using absolute methanol, absolute ethanol, 80% methanol and 80% ethanol and orbital shaker technique in the present study are also in line to those reported by Sultana *et al.*, (2008) for the fruit of *F. religiosa* by using the same four solvents and orbital shaker. Sultana *et al.*, (2008) investigated extraction yields from *F.religiosa* fruit by using absolute methanol, absolute ethanol, 80% methanol and 80% ethanol were 18.9 ± 0.76, 16.9 ± 0.67, 26.4 ± 0.52 and 19.7 ± 0.39 g/100g DW, respectively.

Abdel-Hameed (2009) reported extraction yields for the leaf samples of *F. glomereta*, *F. microcarpa* and *F. virens* equal to 15.32%, 20.25% and 14.11%, respectively by using methanol. The extraction yields determined in the present study for the leaves of *F.infectoria* (*F. virens*) 14.45±0.67g/100g DW and *F. racemosa* (*F. glomereta*) 13.02±0.61 g/100g DW are in close agreement with the values quoted by Abdel-Hameed (2009) but the extraction yield

15.22±0.72 g/100 g DW for the leaves of *F. retusa* were lower than the reported value.

4.2. Influence of the extraction process on total phenolic contents

Different bioactivities and health promoting effects of plants are attributed to the presence of phenolic compounds (Fattouch *et al.*, 2007; Costa *et al.*, 2009). That is why determination of phenolic components in plants remains in the focus of researchers (Djeridane *et al.*, 2006; Wong *et al.*, 2006; Debib *et al.*, 2013). As the total phenolic contents are the main cause of the antioxidant properties of plants hence total phenolic contents must be investigated thoroughly to estimate the antioxidant and other bioactivities of botanical sources (Debib *et al.*, 2013).

In the present study the total phenolic contents (TPC) of the extracts obtained from different samples by using four solvents (100% ethanol, 100% methanol, 80% ethanol and 80% methanol) and three techniques (orbital shaker, sonication and magnetic stirrer) were estimated colorimetrically by using Folin-Ciocalteu reagent (FCR) method. This colorimetric method is widely used for the estimation of TPC from natural sources/plant materials due to its rapidness, simplicity and reproducibility (ksouri *et al.*, 2009). This test is performed in basic medium which supports the formation of phenolate ion by the removal of H^+ from the aromatic ring of phenolic compounds and in this way phenolate ion generated can reduce the molybdenum by donating an electron and blue color is developed. Generally, Gallic acid is used as standard and a standard curve is generated by using different concentrations of standard compound. The results for TPC of fruits, leaves and bark of different species of *Ficus* are listed in table 4.4, table 4.5 and table 4.6, respectively.

The results revealed that the combination of 80% methanol with sonication was the most efficient method and the extracts obtained through this combination constituted significantly ($p<0.05$) higher amounts of total phenolic contents (TPC). Among the solvents, 80% methanol was the most effective solvents and the extracts obtained by the application of 80% contained significantly ($p<0.05$) higher amounts of total phenolic in comparison with the extracts obtained by the application of other solvents used in the present study. The order of extraction efficiency of solvents on the basis of TPC was noted to be 80%methanol > 80% ethanol > 100%methanol

Table 4.4. Effect of extraction procedure on the total phenolic contents (GAE g/100g of dried sample) of extracts obtained from the fruits of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$5.62\pm0.25_{b}^{d}$	$7.04\pm0.31_{b}^{b}$	$5.88\pm0.26_{b}^{c}$	$7.95\pm0.35_{b}^{a}$
	Sonication	$5.77\pm0.25_{a}^{d}$	$7.19\pm0.32_{a}^{b}$	$6.37\pm0.28_{a}^{c}$	$8.22\pm0.36_{a}^{a}$
	Magnetic Stirrer	$5.63\pm0.25_{ab}^{d}$	$7.05\pm0.29_{ab}^{b}$	$6.18\pm0.27_{ab}^{c}$	$8.09\pm0.36_{ab}^{a}$
F. infectoria	Orbital Shaker	$3.28\pm0.23_{b}^{d}$	$3.99\pm0.18_{b}^{c}$	$4.91\pm0.22_{b}^{b}$	$5.28\pm0.23_{b}^{a}$
	Sonication	$3.51\pm0.15_{a}^{d}$	$4.37\pm0.19_{a}^{c}$	$5.11\pm0.22_{a}^{b}$	$5.49\pm0.24_{a}^{a}$
	Magnetic Stirrer	$3.36\pm0.15_{b}^{d}$	$4.08\pm0.18_{b}^{c}$	$4.98\pm0.22_{b}^{b}$	$5.29\pm0.23_{b}^{a}$
F. Racemosa	Orbital Shaker	$5.32\pm0.23_{b}^{c}$	$6.98\pm0.31_{b}^{b}$	$5.58\pm0.25_{b}^{c}$	$8.34\pm0.37_{b}^{a}$
	Sonication	$5.84\pm0.26_{a}^{c}$	$7.86\pm0.35_{a}^{b}$	$5.79\pm0.25_{a}^{c}$	$9.03\pm0.40_{a}^{a}$
	Magnetic Stirrer	$5.40\pm0.24_{b}^{c}$	$7.48\pm0.33_{b}^{b}$	$5.63\pm0.25_{b}^{c}$	$8.54\pm0.38_{b}^{a}$
F. Religiosa	Orbital Shaker	$4.32\pm0.19_{a}^{c}$	$6.31\pm0.28_{a}^{b}$	$4.79\pm0.21_{a}^{c}$	$7.85\pm0.35_{a}^{a}$
	Sonication	$4.49\pm0.20_{a}^{c}$	$6.50\pm0.29_{a}^{b}$	$5.00\pm0.22_{a}^{c}$	$8.13\pm0.36_{a}^{a}$
	Magnetic Stirrer	$4.40\pm0.19_{a}^{c}$	$6.32\pm0.28_{a}^{b}$	$4.79\pm0.21_{a}^{c}$	$8.76\pm0.39_{a}^{a}$
F. retusa	Orbital Shaker	$5.54\pm0.24_{a}^{c}$	$7.40\pm0.33_{a}^{b}$	$5.73\pm0.25_{a}^{c}$	$8.48\pm0.37_{a}^{a}$
	Sonication	$5.66\pm0.25_{a}^{c}$	$7.21\pm0.32_{a}^{b}$	$6.09\pm0.27_{a}^{c}$	$9.58\pm0.42_{a}^{a}$
	Magnetic Stirrer	$5.58\pm0.25_{a}^{c}$	$7.55\pm0.33_{a}^{b}$	$5.89\pm0.26_{a}^{c}$	$8.95\pm0.39_{a}^{a}$

tal phenolic contents (GAE g/100g of dried sample) of extracts

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

>100% ethanol. The extracts obtained by using sonication gave significantly (p<0.05) higher amount of TPC with few exceptions. Sultana *et al.*,(2009) reported that 80% methanol extract contained the highest amount of TPC when compared to other solvents and this finding is in agreement with our present results.

All the fruit samples extract investigated in the present study, showed a fairly good amount of total phenolics but these amounts varied in relation to extraction solvent and the technique employed. This revealed that both extraction solvent as well as the technique has significant effect on the recovery of phenolics from the tested materials. TPC were generally higher in aqueous alcoholic extracts produced by sonication technique. The amount of TPC increased from 4.32±0.19 g/100g DW to 8.76±0.39 g/100g DW and from 5.54±0.24g/100g DW to 9.58±0.42 g/100g DW in the extracts obtained from the fruit of *F. religiosa* and *F. retusa* respectively. Although upper and lower values were significantly (p<0.05) different from each other but the yields obtained by the application of absolute ethanol and methanol were not significantly (p<0.05) different from each other. Total phenolic contents quantified in the extracts obtained from the fruits of *F. religiosa* and *F. retusa* by all the three extraction technique were not significantly (p<0.05) different from one another. For the fruits of *F. racemosa*, TPC increased from 5.32±0.23 to 9.03±0.4 under the influence of different solvents and techniques applied for extraction in the present study. The statistical ranking of solvents on the basis of TPC obtained from the fruits of *F. racemosa* was 80% methanol > 80% ethanol > 100% methanol ≈ 100% ethanol. The TPC obtained by the application of orbital shaker and magnetic stirrer were not significantly (p<0.05) different from each other but the TPC obtained by the application of sonication were significantly (p<0.05) different from the TPC obtained using other two extraction techniques. Total phenolic contents ranged from 3.28±0. 15 to 5.49±0.24 and from 5.62±0.25 to 8.22±0.36 in the fruit extracts of *F. infectoria* and *F.bengalensis*, respectively and for both the samples, TPC obtained by the application of different solvents were significantly (p<0.05) different. For *F. infectoria* TPC obtained by the application of orbital shaker and magnetic stirrer were not significantly different. For all the fruit samples, the highest yields were obtained by the combination of 80% methanol using sonication while the lowest yields were obtained by the combination of 100% ethanol with orbital shaker.

Table 4.5. Effect of extraction procedure on the total phenolic contents (GAE g/100g of dried sample) of extracts obtained from the leaves of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$2.17\pm0.10_{b}^{d}$	$3.41\pm0.15_{b}^{b}$	$2.34\pm0.10_{b}^{c}$	$3.76\pm0.17_{b}^{a}$
	Sonication	$2.23\pm0.10_{a}^{d}$	$3.52\pm0.15_{a}^{b}$	$2.41\pm0.11_{a}^{c}$	$3.88\pm0.17_{a}^{a}$
	Magnetic Stirrer	$2.19\pm0.12_{b}^{d}$	$3.44\pm0.15_{b}^{b}$	$2.37\pm0.10_{b}^{c}$	$3.77\pm0.17_{b}^{a}$
F. infectoria	Orbital Shaker	$3.35\pm0.15_{b}^{c}$	$3.60\pm0.16_{b}^{b}$	$3.87\pm0.17_{b}^{b}$	$5.21\pm0.23_{b}^{a}$
	Sonication	$3.70\pm0.16_{a}^{c}$	$4.00\pm0.18_{a}^{b}$	$3.95\pm0.17_{a}^{b}$	$5.36\pm0.24_{a}^{a}$
	Magnetic Stirrer	$3.36\pm0.15_{b}^{c}$	$3.63\pm0.16_{b}^{b}$	$3.89\pm0.17_{b}^{b}$	$5.22\pm0.23_{b}^{a}$
F. Racemosa	Orbital Shaker	$2.75\pm0.12_{a}^{d}$	$4.07\pm0.18_{a}^{b}$	$3.39\pm0.15_{a}^{c}$	$4.62\pm0.20_{a}^{a}$
	Sonication	$2.82\pm0.12_{a}^{d}$	$4.21\pm0.19_{a}^{b}$	$3.48\pm0.15_{a}^{c}$	$5.41\pm0.24_{a}^{a}$
	Magnetic Stirrer	$2.76\pm0.12_{a}^{d}$	$4.08\pm0.18_{a}^{b}$	$3.41\pm0.15_{a}^{c}$	$4.94\pm0.22_{a}^{a}$
F. Religiosa	Orbital Shaker	$1.35\pm0.06_{b}^{d}$	$1.71\pm0.08_{b}^{b}$	$1.48\pm0.07_{b}^{c}$	$2.21\pm0.09_{b}^{a}$
	Sonication	$1.51\pm0.07_{a}^{d}$	$1.74\pm0.08_{a}^{b}$	$1.61\pm0.07_{a}^{c}$	$2.28\pm0.10_{a}^{a}$
	Magnetic Stirrer	$1.48\pm0.07_{ab}^{d}$	$1.72\pm0.08_{ab}^{b}$	$1.5\pm0.07_{ab}^{c}$	$2.23\pm0.10_{ab}^{a}$
F. retusa	Orbital Shaker	$3.72\pm0.16_{b}^{d}$	$5.04\pm0.22_{b}^{b}$	$4.54\pm0.20_{b}^{c}$	$5.24\pm0.23_{b}^{a}$
	Sonication	$3.88\pm0.17_{a}^{d}$	$5.21\pm0.23_{a}^{b}$	$4.7\pm0.21_{a}^{c}$	$5.5\pm0.24_{a}^{a}$
	Magnetic Stirrer	$3.77\pm0.17_{b}^{d}$	$5.06\pm0.22_{b}^{b}$	$4.55\pm0.20_{b}^{c}$	$5.25\pm0.23_{b}^{a}$

tal phenolic contents (GAE g/100g of dried sample) of extracts

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

Total phenolic contents (TPC) present in the extracts of leaf samples of selected species of *Ficus* increased appreciably under the influence of different combinations of extraction solvent and technique applied. Under optimum extraction system, the quantity of TPC in the extract obtained from the leaves of *F. racemosa* improved from 2.75±0.12 g/100 g DW to 5.41±0.24 g/100 g DW (an increment of 96.7%) and these amounts were significantly (p<0.05) different from each other. All the three extraction techniques have no significant (p<0.05) effect on the amount of TPC obtained from the leaves of *F. racemosa* but all the four solvents yield significantly (p<0.05) different amounts of TPC from the leaves of *F. racemosa*. With regard to effect of extraction system, the enhancement in the quantity of total phenolic contents from the leaves of
F. bengalensis, F. religiosa, *F.infectoria* and *F.retusa* were 78.8% (from 2.17±0.10 to 3.88±0.17), 68.9% (from 1.35±0.06 to 2.28±0.1), 60% (from 3.35±0.15 to 5.36±0.24) and 47.8% (from 3.72±0.16 to 5.5±0.24), respectively. All the upper and lower values for each leaf sample were significantly (p<0.05) different from each other. Significantly (p<0.05) different amounts of TPC were obtained for the leaf samples of *F. bengalensis, F. religiosa* and *F.retusa* under the influence of different solvents used but for *F. infectoria,* the amounts of TPC obtained by the use of absolute methanol and absolute ethanol were not significantly (p<0.05) different. On the other hands, the amounts of TPC obtained from the leaves of *F. bengalensis, F.infectoria* and *F.retusa* by the use of orbital shaker and magnetic stirrer were not significantly (p<0.05) different from each other. As far as variation among leaf samples of the selected species of *Ficus* is concerned, the leaves of *F.retusa* contained the highest amount of TP while leaves of *F .religiosa* offered the lowest amount regardless of the combination of extraction solvent and technique employed in the present analysis.

The total phenolic contents of the bark samples of the selected species of *Ficus* were also influenced noticeably by the application of different extraction solvents and techniques. The total phenolic contents in the extract of the bark of *F. bengalensis* increased from 3.59±0.15 g/100 g DW to 6.83±0.29 g/100 g DW while applying the least efficient combination of solvent and technique (absolute ethanol and orbital shaker) to the most efficient combination (80% methanol and sonication) applied. Similarly, different combinations of solvent and technique on the bark sample of *F. retusa* increased the TPC from 3.84±0.16 to 7.15±0.3 g/100 g DW. And this enhancement was equal to 86.2%. The improvements in the amounts of TPC present in the extracts obtained from the barks of *F .religiosa, F.racemosa* and *F.infectoria* were 73% (from

Table 4.6. Effect of extraction procedure on the total phenolic contents (GAE g/100g of dried sample) of extracts obtained from the bark of selected species of Ficus

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$3.59\pm0.15_{b}^{d}$	$5.35\pm0.22_{b}^{b}$	$4.02\pm0.17_{b}^{c}$	$6.69\pm0.28_{b}^{a}$
	Sonication	$3.68\pm0.15_{a}^{d}$	$5.42\pm0.23_{a}^{b}$	$4.11\pm0.17_{a}^{c}$	$6.83\pm0.29_{a}^{a}$
	Magnetic Stirrer	$3.60\pm0.15_{b}^{d}$	$5.37\pm0.23_{b}^{b}$	$4.02\pm0.17_{b}^{c}$	$6.70\pm0.28_{b}^{a}$
F. infectoria	Orbital Shaker	$3.73\pm0.16_{b}^{d}$	$4.64\pm0.19_{b}^{b}$	$3.87\pm0.16_{b}^{c}$	$5.34\pm0.22_{b}^{a}$
	Sonication	$3.82\pm0.16_{a}^{d}$	$4.77\pm0.20_{a}^{b}$	$3.97\pm0.17_{a}^{c}$	$5.43\pm0.23_{a}^{a}$
	Magnetic Stirrer	$3.74\pm0.16_{b}^{d}$	$4.64\pm0.19_{b}^{b}$	$3.89\pm0.16_{b}^{c}$	$5.36\pm0.23_{b}^{a}$
F. Racemosa	Orbital Shaker	$4.80\pm0.20_{b}^{d}$	$6.51\pm0.27_{b}^{b}$	$4.86\pm0.20_{b}^{c}$	$7.23\pm0.31_{b}^{a}$
	Sonication	$4.92\pm0.21_{a}^{d}$	$6.68\pm0.28_{a}^{b}$	$4.99\pm0.21_{a}^{c}$	$7.42\pm0.31_{a}^{a}$
	Magnetic Stirrer	$4.81\pm0.22_{b}^{d}$	$6.53\pm0.27_{b}^{b}$	$4.87\pm0.20_{b}^{c}$	$7.23\pm0.30_{b}^{a}$
F. Religiosa	Orbital Shaker	$3.90\pm0.16_{b}^{d}$	$5.44\pm0.23_{b}^{b}$	$4.14\pm0.17_{b}^{c}$	$6.56\pm0.28_{b}^{a}$
	Sonication	$4.03\pm0.17_{a}^{d}$	$5.59\pm0.23_{a}^{b}$	$4.25\pm0.18_{a}^{c}$	$6.75\pm0.28_{a}^{a}$
	Magnetic Stirrer	$3.94\pm0.17_{b}^{d}$	$5.45\pm0.23_{b}^{b}$	$4.14\pm0.17_{b}^{c}$	$6.57\pm0.28_{b}^{a}$
F. retusa	Orbital Shaker	$3.84\pm0.16_{b}^{d}$	$5.66\pm0.24_{b}^{b}$	$4.27\pm0.18_{b}^{c}$	$7.02\pm0.29_{b}^{a}$
	Sonication	$4.00\pm0.17_{a}^{d}$	$5.73\pm0.24_{a}^{b}$	$4.41\pm0.19_{a}^{c}$	$7.15\pm0.31_{a}^{a}$
	Magnetic Stirrer	$3.84\pm0.16_{b}^{d}$	$5.67\pm0.24_{b}^{b}$	$4.28\pm0.18_{b}^{c}$	$7.03\pm0.30_{b}^{a}$

tal phenolic contents (GAE g/100g of dried sample) of extracts

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

3.9±0.16 to 6.75±0.28), 54.6% (from 4.8±0.2 to 7.42±0.31) and 45.6% (from 3.73±0.16 to 5.43±0.23), respectively. The lowest and the highest concentrations of TPC of each bark sample mentioned above were significantly ($p<0.05$) different from each other. Total phenolic contents obtained by the use of different solvents (100% ethanol, 100% methanol, 80% ethanol and 80% methanol) from the bark sample of each specie were significantly ($p<0.05$) different from one another but the total phenolic contents obtained by the use of orbital shaker and magnetic stirrer were not significantly ($p<0.05$) different from each other although TPC obtained by the use of sonication were significantly ($p<0.05$) different from the extracts obtained by the use of other two techniques. The lowest concentrations of TPC were recorded in the extracts produced by using absolute ethanol and orbital shaking technique whereas the highest amounts of TPC were extracted by the application of 80%methanol and sonication. Among the bark samples, the bark of *F. racemosa* offered the highest TPC with all the combinations of solvent and technique applied for extraction while the extract from the bark of *F. bengalensis* contained the least amount of TP when the solvent was absolute ethanol and shaker as the extraction technique.

In a previous study, Sultana *et al.*, (2009) reported 5.34 ± 0.36 g/100 g DW of TPC in fruits of *F.religiosa* when solvent was 80% methanol with orbital shaker as extraction technique. The investigated amounts of TPC by Sultana *et al.*, (2009) in fruits of *F. religiosa* were lower than that recorded amount of TPC (9.2±0.4 GAE g/100 g of DW) in the present study using the same solvent and the extraction technique. On the other hand, the contents of TPC reported in the fruits of *F. glomerata* (170 GAE mg/g of dry extract) by Verma *et al.*, (2010) using 50% methanol was noted to be higher than our present results. Ao *et al.*, (2008) reported total phenolic contents equal to 179 mg GAE/ g extract from the fruit of *F. microcarpa* using 100% methanol. Chen *et al.*, (2013) studied the effect of different factors including solvent concentration, extraction time, temperature and extraction cycles on total phenolic contents and antioxidant activities of leaves of *F. virens* (*F. infectoria*) and reported minimum and maximum value of TPC equal to 3.06±0.4 and 4.95±0.6% at different concentrations of ethanol. The values reported for *F. infectoria* in our study also fall within this range i.e. from 3.7±0.17 to3.95±0.16 GAE g/ 100 g DW for the same solvent extracts. Ao *et al.*, (2008) reported TPC from the leaves of *F .microcarpa* (*F. retusa*) to be 127 mg GAE/ g extract. The reported amount is below than the amounts 5.41±0.24 GAE g/100g DW (271.4 mg GAE/g extract) determined in the present analysis. Melinda *et al.*, (2010) conducted a study to compare the antioxidant activities of the methanol extracts from the leaf of *F. religiosa*, *Chromolaena odorata, Cyanodon dactylon* and *Tridax procumbens* and reported total phenolic contents equal to 235±4.41GAE µg/mg. The reported amount is much higher than our calculated value of TPC 1.61±0.07 GAE g/ 100g DW for *F. religiosa* with the same extraction solvent.

Manian *et al*., (2008) reported total phenolic contents equal to 59.6 % of extract obtained from the stem bark of *F. racemosa* which was similar to the TPC determined (4.99±0.22GAE g/100g DW; 60.4% of extract) in the present study with same solvent (100% methanol). In agreement with the present results, Ao *et al.,* (2008) determined the amount of TP in the stem bark of *F .microcarpa* (*F. retusa*) to be 237 mg GAE/ g extract. Anandjiwala *et al*., (2008) reported TPC by using 100% methanol as solvent in the stem bark of *F. bengalensis, F. glomerata, F. religiosa* and *F. virens* (*F. infectoria)* equal to 3.59±0.01, 10.80±0.23, 7.89±0.01, 3.84±0.03 % (w/w), respectively. These results reported for stem bark of *F. bengalensis* and *F. virens* (*F. infectoria)* are close to our data 4.02±0.18 GAE g/ 100 g DW for *F. bengalensis* and 3.89±0.17 GAE g/ 100 g DW for *F.infectoria* but the reported amount for bark of *F. glomerata* and *F. religiosa* are higher than our results for the stem bark of *F.racemosa* (*F. glomerata*) (4.99±0.22 GAE g/ 100g DW) and for the stem bark of *F. religiosa* (4.25±0.19 GAE g/ 100g DW).

There are many factors which affect the investigation of different bioactive components and bioactivities of medicinal plants and other botanical sources. These factors include extraction technique, solvent, solvent to solid ratio, temperature, duration of extraction (Harborne and Williams, 2000; Shahidi and Naczk, 2004). There are published reports which show that total phenolics may vary not only within different sources of plants but also with in different parts of the same plant (Veberic *et al.*, 2008)**.** Numerical values for TPC are different in different published reports but most of the researchers agreed that extracts obtained by using polar solvents contain higher total phenolic contents (Bucic-Kojic *et al*., 2009; Spigno *et al*., 2007).

4.3. Influence of extraction process on total flavonoids content

The results obtained for the total flavonoid contents (TFC) are presented in table 4.7 for fruits, in table 4.8 for leaves and in table 4.9 for barks. All the samples in the present study were extracted for their TFC by using three extraction techniques (orbital shaker, sonication and magnetic stirring) and four solvents (absolute ethanol, absolute methanol, 80% ethanol and 80% methanol). The results were presented as catechin equivalent (CE) grams per 100 grams of dry weight (DW).

Among all the tested samples, the highest amount of total flavonoids (TF) was extracted from the fruits of *F.retusa* with sonication extraction technique and 80% methanol as solvent. Comparison of the results showed that sonication extraction technique was the most efficient than other two extraction techniques used for the recovery of TF while magnetic stirring was more efficient than orbital shaker. In most of the cases, amounts of total flavonoid obtained by the application of magnetic stirrer and orbital shaker were not significantly ($p<0.05$) different from each other. If we compare the efficiency of the solvent for the extraction of TF then order of efficiency in most of the samples was 80% methanol > 80% ethanol > absolute methanol >absolute ethanol. These trends observed in the present study regarding the efficiency of extraction solvent for recovery of total flavonoids from different parts of *Ficus* species, are in close agreement to those recorded by Sultana *et al.,* (2009) and Zahid *et al.,* (2012) for extraction of flavonoids from different botanical materials using the same solvents for extraction.

In the present study different solvents and techniques exhibited significant ($P<0.05$) effect on the yield of total flavonoids from the fruits of *Ficus* species. With regard to extraction solvent and techniques, among fruit samples analyzed, the highest amount of total flavonoids (5.88±0.28 CE g/100g DW) was extracted from *F. retusa* fruit with 80% methanol and sonication as extraction system. Among other *Ficus* fruits, the range of TFC for fruit of *F. bengalensis* was from 1.55±0.07 CE g/100g DW to 4.35±0.21 CE g/100g DW, for *F. racemosa* TFC ranged from 1.37±0.07 CE g/100g DW to 2.77±0.13 CE g/100g DW, for *F. religiosa* 1.29±0.06 CE g/100g DW to 2.64±0.13 CE g/100g DW and for *F.infectoria* 1.55±0.07 CE g/100g DW to 2.69±0.13 CE g/100g DW. For the fruits of *F. bengalensis, F.infectoria, F. racemosa* and *F. retusa*, all the four solvents applied yielded significantly ($p<0.05$) different amounts of total flavonoids but for the fruits of *F. religiosa*, the amounts of total flavonoids obtained by the application absolute ethanol and absolute methanol were not significantly ($p<0.05$) different from each other. In case of *F. racemosa* and *F. religiosa*, the amounts of total flavonoids obtained by the application of all the three extraction techniques (orbital shaker, sonication and magnetic stirrer) were not significantly ($p<0.05$) different from each other. Total flavonoids obtained by the application of orbital shaker and magnetic stirrer from the fruits of *F.*

Table 4.7. Effect of extraction procedure on the total flavonoids (CE g/100g of dried sample) of extracts obtained from the fruits of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$1.55{\pm}0.07_b^d$	$1.75{\pm}0.08_b^c$	$2.10{\pm}0.10_b^b$	$4.27{\pm}0.20_b^a$
	Sonication	$1.65{\pm}0.08_a^d$	$1.83{\pm}0.09_a^c$	$2.14{\pm}0.10_a^b$	$4.35{\pm}0.21_a^a$
	Magnetic Stirrer	$1.56{\pm}0.07_b^d$	$1.76{\pm}0.08_b^c$	$2.11{\pm}0.11_b^b$	$4.28{\pm}0.20_b^a$
F. infectoria	Orbital Shaker	$1.55{\pm}0.07_b^d$	$2.30{\pm}0.11_b^b$	$1.66{\pm}0.08_b^c$	$2.41{\pm}0.12_b^a$
	Sonication	$1.60{\pm}0.08_a^d$	$2.47{\pm}0.12_a^b$	$1.86{\pm}0.09_a^c$	$2.69{\pm}0.13_a^a$
	Magnetic Stirrer	$1.56{\pm}0.07_b^d$	$2.31{\pm}0.11_b^b$	$1.67{\pm}0.08_b^c$	$2.44{\pm}0.12_b^a$
F. Racemosa	Orbital Shaker	$1.37{\pm}0.07_a^d$	$2.01{\pm}0.11_a^b$	$1.78{\pm}0.09_a^c$	$2.58{\pm}0.12_a^a$
	Sonication	$1.40{\pm}0.07_a^d$	$2.20{\pm}0.11_a^b$	$1.80{\pm}0.09_a^c$	$2.77{\pm}0.13_a^a$
	Magnetic Stirrer	$1.42{\pm}0.07_a^d$	$2.09{\pm}0.10_a^b$	$1.77{\pm}0.08_a^c$	$2.31{\pm}0.11_a^a$
F. Religiosa	Orbital Shaker	$1.29{\pm}0.06_a^c$	$1.94{\pm}0.09_a^b$	$1.18{\pm}0.06_a^c$	$2.58{\pm}0.12_a^a$
	Sonication	$1.37{\pm}0.07_a^c$	$2.00{\pm}0.1_a^b$	$1.20{\pm}0.06_a^c$	$2.64{\pm}0.13_a^a$
	Magnetic Stirrer	$1.97{\pm}0.09_a^c$	$1.97{\pm}0.09_a^b$	$1.18{\pm}0.06_a^c$	$2.60{\pm}0.12_a^a$
F. retusa	Orbital Shaker	$1.10{\pm}0.05_b^d$	$2.74{\pm}0.13_b^b$	$1.26{\pm}0.06_b^c$	$5.76{\pm}0.28_b^a$
	Sonication	$1.15{\pm}0.05_a^d$	$2.83{\pm}0.14_a^b$	$1.27{\pm}0.06_a^c$	$5.88{\pm}0.28_a^a$
	Magnetic Stirrer	$1.12{\pm}0.05_b^d$	$2.79{\pm}0.13_b^b$	$1.27{\pm}0.06_b^c$	$5.80{\pm}0.28_b^a$

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

bengalensis, F.infectoria and *F. retusa* were not significantly ($p<0.05$) different from each other although TFC obtained with the help of sonication from the fruits of these plants were significantly ($p<0.05$) different from the TFC obtained by the use of orbital shaker and magnetic stirrer. In all these fruit samples, the highest amounts of TFC were obtained when the solvent was 80% methanol and the technique was sonication, whereas, the lowest values of TFC were recorded for samples extracted with 100% ethanol and technique was orbital shaker. Such variation in the extractable amounts of TFC in relation to different solvents, techniques and fruits might be in due part to the genetic makeup of the species as well as to the extent of solvent polarity and efficacy of extraction technique employed.

The data obtained for the TFC obtained from the leaves of *Ficus* species investigated explored that all the leaf sample showed similar trends with regard to technique i.e. sonication extraction offered the highest amounts followed by magnetic stirring and orbital shaker. Although TFC obtained by the application of orbital shaker and magnetic stirrer were not significantly ($p<0.05$) different from each other in case of *F .infectoria, F. racemosa* and *F. religiosa*. Statistical ranking of the solvents on the basis of their ability to extract flavonoids from the leaves of investigated species was found to be 80% methanol> 80% ethanol> 100% methanol > 100% ethanol for all the leaves samples except for the leaves of *F .infectoria* for which this order was 80% methanol> 80% ethanol> 100% ethanol> 100% methanol. Significantly ($p<0.05$) different amounts of total flavonoids were obtained from the leaves of tested species under the influence of different solvents applied in the present study. Total flavonoid contents of leaves of the same species varied over a wide range in relation to different extraction technique and solvent employed. This variation of TFC for the same sample indicated significant effect of extraction solvent and technique on the extractable amount of total flavonoids. In relation to efficacy of extraction solvent and techniques, for *F. religiosa* the lowest value of TFC was 0.31±0.01and the highest was 1.1±0.05 (an increase of 254.8%), for *F.bengalensis* the lowest amount of TF was 0.18±0.01 and the highest was 0.45±0.02 (an increase of 150%), for the leaf of *F. racemosa* the lowest quantity of TF was 0.97±0.05 and the highest was 2.21±0.11 (an increase of 127.8%), for the leaf of *F. infectoria* the lowest amount of TF was equal to 1.42±0.07 and the highest was 1.92±0.09 (an increase of 35.2%) and for the leaf samples of *F. retusa* the lowest value of TF was 3.32±0.16 and the highest was 4.18±0.2 CE g/100g DW (an increase of 26%). The lowest amount and the highest amount mentioned above

Table 4.8. Effect of extraction procedure on the total flavonoids (CE g/100g of dried sample) of extracts obtained from the leaves of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
F. bengalensis	Orbital Shaker	$0.18\pm0.01b^{d}$	$0.30\pm0.02_{b}^{b}$	$0.21\pm0.01_{b}^{c}$	$0.42\pm0.02_{b}^{a}$
	Sonication	$0.19\pm0.01_{a}^{d}$	$0.33\pm0.02_{a}^{b}$	$0.21\pm0.01_{a}^{c}$	$0.45\pm0.02_{a}^{a}$
	Magnetic Stirrer	$0.18\pm0.01_{ab}^{d}$	$0.32\pm0.02_{ab}^{b}$	$0.20\pm0.01_{ab}^{c}$	$0.43\pm0.02_{ab}^{a}$
F. infectoria	Orbital Shaker	$1.59\pm0.08_{b}^{c}$	$1.64\pm0.08_{b}^{b}$	$1.42\pm0.07_{b}^{d}$	$1.85\pm0.09_{b}^{a}$
	Sonication	$1.61\pm0.08_{a}^{c}$	$1.67\pm0.08_{a}^{b}$	$1.46\pm0.07_{a}^{d}$	$1.92\pm0.09_{a}^{a}$
	Magnetic Stirrer	$1.60\pm0.08_{b}^{c}$	$1.65\pm0.08_{b}^{b}$	$1.43\pm0.07_{b}^{d}$	$1.86\pm0.09_{b}^{a}$
F. Racemosa	Orbital Shaker	$0.97\pm0.05_{b}^{d}$	$1.38\pm0.07_{b}^{b}$	$1.13\pm0.05_{b}^{c}$	$2.18\pm0.1_{b}^{a}$
	Sonication	$1.00\pm0.05_{a}^{d}$	$1.41\pm0.07_{a}^{b}$	$1.14\pm0.05_{a}^{c}$	$2.21\pm0.11_{a}^{a}$
	Magnetic Stirrer	$0.97\pm0.05_{b}^{d}$	$1.39\pm0.07_{b}^{b}$	$1.13\pm0.05_{b}^{c}$	$2.19\pm0.10_{b}^{a}$
F. Religiosa	Orbital Shaker	$0.31\pm0.01_{b}^{d}$	$1.01\pm0.05_{b}^{b}$	$0.32\pm0.02_{b}^{c}$	$1.06\pm0.05_{b}^{a}$
	Sonication	$0.32\pm0.02_{a}^{d}$	$1.05\pm0.05_{a}^{b}$	$0.34\pm0.02_{a}^{c}$	$1.10\pm0.05_{a}^{a}$
	Magnetic Stirrer	$0.31\pm0.01_{b}^{d}$	$1.03\pm0.05_{b}^{b}$	$0.33\pm0.02_{b}^{c}$	$1.07\pm0.03_{b}^{a}$
F. retusa	Orbital Shaker	$3.32\pm0.16_{c}^{d}$	$3.84\pm0.18_{c}^{b}$	$3.74\pm0.18_{c}^{c}$	$4.07\pm0.19_{c}^{a}$
	Sonication	$3.41\pm0.16_{a}^{d}$	$3.92\pm0.19_{a}^{b}$	$3.83\pm0.18_{a}^{c}$	$4.18\pm0.20_{a}^{a}$
	Magnetic Stirrer	$3.35\pm0.16_{b}^{d}$	$3.84\pm0.18_{b}^{b}$	$3.77\pm0.18_{b}^{c}$	$4.10\pm0.20_{b}^{a}$

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

for each specie were significantly different from each other. This data showed that solvent and different extraction techniques have proliferative effect on the TFC of leaf sample of *Ficus* species evaluated in the present study. As far as the occurrence of total flavonoids among leaf samples was concerned, regardless of the solvent and extraction technique employed, it was evident that *F.retusa* has the highest amount of TFC among all the tested species.

The results obtained for the total flavonoid contents of the barks of the selected species of *Ficus* also followed the similar trends related to the effectiveness of extraction technique and solvents employed. The combination of 80% methanol with sonication was the most effective for the extraction of TF from bark samples followed by magnetic stirring and 80% methanol and then orbital shaker and 80% methanol. The analysis of bark samples revealed that extraction technique and solvent has appreciable effects on the yield of TFC. As function of extraction solvent and techniques employed, the amount of TFC for bark samples varied from 1.14±0.05 to 2.39±0.11 CE g/100g DW for *F.bengalensis,* 1.38±0.07 to 2.02±0.1 CE g/100g DW for *F. racemosa*, 1.31±0.06 to 1.94±0.09 CE g/100g DW for *F. retusa*, 1±0.03 to 1.43±0.07 CE g/100g DW for *F. religiosa*, and 0.99±0.04 to 1.32±0.06 CE g/100g DW for *F. infectoria*. All the higher amounts from the bark of each specie was obtained by the application of 80% methanol and significantly ($p<0.05$) different amounts of TF obtained under the influence of different solvents (80% methanol, 80% ethanol, 100% methanol and 100% ethanol) from each species. As for as effect of techniques is concerned, TFC obtained from all the tested species by the application of orbital shaker and magnetic stirrer were not significantly ($p<0.05$) different from each other but the TFC obtained from all the species by the application of sonication were significantly ($p<0.05$) different from the TFC obtained by the application of orbital shaker and magnetic stirrer. When compared the TFC among the bark samples of different *Ficus* species investigated then the highest TFC was found in *F. bengalensis* (2.42±0.12) with the application of 80% methanol with sonication while the lowest amount of TFC (0.98±0.05) when absolute ethanol and magnetic stirrer was applied for extraction. This indicates that extraction solvent due to their polarity has significant effect on the recovery of TFC from the bark samples. This advocates that the nature and polarity of the extraction solvent should be compatible to the chemical nature of the extractable phytochemicals.

Table 4.9. Effect of extraction procedure on the total flavonoids (CE g/100g of dried sample) of extracts obtained from the bark of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
F. bengalensis	Orbital Shaker	$1.12{\pm}0.05_b^d$	$1.66{\pm}0.08_b^b$	$1.51{\pm}0.07_b^c$	$2.36{\pm}0.11_b^a$
	Sonication	$1.17{\pm}0.06_a^d$	$1.71{\pm}0.08_a^b$	$1.55{\pm}0.07_a^c$	$2.42{\pm}0.12_a^a$
	Magnetic Stirrer	$1.14{\pm}0.05_b^d$	$1.67{\pm}0.08_b^b$	$1.52{\pm}0.07_b^c$	$2.39{\pm}0.11_b^a$
F. infectoria	Orbital Shaker	$0.99{\pm}0.04_b^d$	$1.22{\pm}0.06_b^b$	$1.01{\pm}0.05_b^c$	$1.32{\pm}0.06_b^a$
	Sonication	$1.02{\pm}0.05_a^d$	$1.25{\pm}0.06_a^b$	$1.11{\pm}0.05_a^c$	$1.34{\pm}0.06_a^a$
	Magnetic Stirrer	$0.98{\pm}0.05_b^d$	$1.22{\pm}0.06_b^b$	$1.04{\pm}0.05_b^c$	$1.33{\pm}0.06_b^a$
F. Racemosa	Orbital Shaker	$1.36{\pm}0.07_b^d$	$1.87{\pm}0.09_b^b$	$1.42{\pm}0.07_b^c$	$2.00{\pm}0.10_b^a$
	Sonication	$1.42{\pm}0.07_a^d$	$1.93{\pm}0.09_a^b$	$1.46{\pm}0.07_a^c$	$2.11{\pm}0.10_a^a$
	Magnetic Stirrer	$1.38{\pm}0.07_b^d$	$1.88{\pm}0.09_b^b$	$1.43{\pm}0.07_b^c$	$2.02{\pm}0.10_b^a$
F. Religiosa	Orbital Shaker	$1.00{\pm}0.03_b^d$	$1.25{\pm}0.06_b^b$	$1.04{\pm}0.05_b^c$	$1.39{\pm}0.07_b^a$
	Sonication	$1.03{\pm}0.05_a^d$	$1.29{\pm}0.06_a^b$	$1.07{\pm}0.05_a^c$	$1.43{\pm}0.07_a^a$
	Magnetic Stirrer	$1.01{\pm}0.05_b^d$	$1.26{\pm}0.06_b^b$	$1.04{\pm}0.05_b^c$	$1.43{\pm}0.07_b^a$
F. retusa	Orbital Shaker	$1.30{\pm}0.06_b^d$	$1.52{\pm}0.07_b^b$	$1.38{\pm}0.07_b^c$	$1.91{\pm}0.09_b^a$
	Sonication	$1.34{\pm}0.06_a^d$	$1.59{\pm}0.08_a^b$	$1.44{\pm}0.07_a^c$	$2.00{\pm}0.1_a^a$
	Magnetic Stirrer	$1.32{\pm}0.06_b^d$	$1.54{\pm}0.07_b^b$	$1.40{\pm}0.07_b^c$	$1.93{\pm}0.09_b^a$

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

In agreement with our present findings, Sultana *et al.*, (2009) investigated that 80% methanol extracted the highest contents of TF, as compared to 80% ethanol, 100% methanol and 100% ethanol, from different parts of medicinal plants. In a previous study, total flavonoid contents from the fruits of *F. religiosa* reported by Sultana *et al.*, (2009) were 1.28 ± 0.04 CE g/100 g DW with 100% ethanol as solvent, 2.03 ± 0.06 CE g/100 g DW with 80% ethanol as solvent, 2.16 ± 0.08 CE g/100 g DW with 100% methanol as solvent and 3.77 ±0.1 CE g/100 g DW with 80% methanol as solvent using orbital shaker as extraction technique. These reported values are in agreement with the values determined in the present study when the solvent was 100% or 80% ethanol with same extraction technique but higher than present data when the solvent was 100% methanol or 80% methanol. In another report, sum of TFC for the two fractions (ethyl acetate and butanol) from the leaves of *F. virens* (*F. infectoria*) reported by Abdul-Hameed *et al.*, (2009) was equal to 101.14 rutin equivalent mg/ g of extract. The reported value was quite close to the TFC determined (101 CE mg/g of extract) for the leaf sample of *F. infectoria* in the present work. Krishanti *et al.*, (2010) reported TFC from the leaves of *F. religiosa* equal to 93.67±6 CE mg/g DW which was much higher than our calculated value 1.03±0.05 CE g/100 g DW. However, the reported value 3.51CE mg/g DW of TFC for the leaf of *F. racemosa* by Shi *et al.*, (2011) was much lower than our finding 0.97±0.05 CE g/100 g DW. Konyalioglu *et al.*, (2005) reported TFC for the leaves of *F. carica* equal to 1.152 ±0.021% by using methanol as extraction solvent and this value was comparable with our present values for the leaf samples of *F. infectoria* (1.42±0.07) and *F. racemosa* (1.13±0.05) but higher than the TFC of leaves of *F. bengalensis* (0.21±0.01) and *F. religiosa* (0.32±0.02) and lower than the TFC of leaves of *F.retusa* (3.74±0.18) using the same extraction solvent. Data obtained in this study clearly indicated that different parts of *Ficus* species analyzed have appreciable amount of TF, however, the amount of these phenolic compounds was strongly effected by different extraction solvents and techniques employed.

4.4. Influence of extraction process on DPPH radical scavenging activity

Phenolic compounds can exhibit their antioxidant activity through different mechanisms, scavenging of free radical is one of them. Free radicals can attack on biomolecules present in body and food; hence the evaluation of radical scavenging activity of plant extracts is pivotal to establish their antioxidant activity. DPPH radical scavenging assay is preferred for the evaluation of radical scavenging activity of plant extracts over other methods developed for this purposes because of its rapidness, simplicity and due to the stability of DPPH radical (Anandjiwala *et al.*, 2008; Layina-

Pathirana and Shahidi, 2005). In the present study, this method was used for the determination of radical scavenging activity of the samples and the results were reported as IC_{50} (µg/mL) value. IC_{50} value is the amount of extract which can inhibit/scavenge 50% concentration of DPPH free radicals. Lower IC_{50} value means higher ability of the extract to scavenge the DPPH free radical. Results obtained after the evaluation of DPPH radical scavenging activity of different parts (fruit, leaf and bark) of selected species of *Ficus* were presented in table 4.10 to table 4.12.

All the fruit samples of selected species of *Ficus* showed appreciable DPPH radical scavenging activity. Comparison of results revealed that DPPH radical scavenging activity of the samples investigated also showed mainly the similar trends as observed for the other antioxidant assays. The extract obtained by using 80% methanol proved to be the most effective DPPH radical scavenger among other solvents extracts tested. The efficiency of the solvents towards extraction of potent radical scavengers was of the order: 80% methanol> 80% ethanol>100% methanol> 100% ethanol. Similarly, *Ficus* extracts obtained by using sonication extraction technique scavenged DPPH radical more efficiently as compared to the extracts obtained by using orbital shaking or magnetic stirring as extraction technique provided that the solvent was the same. The extracts obtained by using magnetic stirring came on 2^{nd} and orbital shaker on 3^{rd} position according to their efficiency towards scavenging DPPH radicals. In most of the cases, DPPH radical scavenging activities of the extracts obtained by these two techniques were not significantly ($p<0.05$) different from each other.

Among the extraction solvents and techniques employed for extraction, the extracts produced by the combination of sonication extraction technique with 80% methanol exhibited the highest DPPH free radical scavenging capacity whereas those obtained by the combination of orbital shaker with 100% ethanol as extraction system proved to be the least effective in scavenging the free radicals. Our results about the DPPH radical scavenging efficiency of different solvent extracts of *Ficus* species were in agreement with the results published by Sultan *et al.*, (2009) who also evaluated the DPPH radical potential of same solvent extracts of selected medicinal plants.

Table 4.10. Effect of extraction procedure on DPPH radical scavenging activity (IC_{50} µg/mL) of the extracts obtained from the fruits of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
F. bengalensis	Orbital Shaker	$36.40\pm2.37_{a}^{a}$	$27.30\pm1.99_{a}^{c}$	$29.60\pm2.08_{a}^{b}$	$23.36\pm0.65_{a}^{d}$
	Sonication	$34.65\pm2.3_{b}^{a}$	$25.75\pm1.92_{b}^{c}$	$28.55\pm2.04_{b}^{b}$	$20.60\pm0.53_{b}^{d}$
	Magnetic Stirrer	$36.25\pm2.36_{a}^{a}$	$27.15\pm1.98_{a}^{c}$	$29.40\pm2.07_{a}^{b}$	$21.95\pm0.59_{a}^{d}$
F. infectoria	Orbital Shaker	$57.25\pm2.82_{a}^{a}$	$51.60\pm2.55_{a}^{c}$	$54.25\pm2.70_{a}^{b}$	$49.50\pm2.08_{a}^{d}$
	Sonication	$55.00\pm3.02_{b}^{a}$	$50.50\pm2.45_{b}^{c}$	$53.00\pm2.65_{b}^{b}$	$46.15\pm1.94_{b}^{d}$
	Magnetic Stirrer	$55.60\pm3.05_{ab}^{a}$	$51.45\pm2.54_{ab}^{c}$	$54.25\pm2.70_{ab}^{b}$	$47.15\pm2.06_{ab}^{d}$
F. Racemosa	Orbital Shaker	$49.70\pm2.51_{a}^{a}$	$42.90\pm1.8_{a}^{b}$	$45.60\pm2.29_{a}^{b}$	$34.75\pm1.04_{a}^{c}$
	Sonication	$46.30\pm2.36_{a}^{a}$	$40.70\pm1.63_{a}^{b}$	$43.05\pm2.23_{a}^{b}$	$32.30\pm0.94_{a}^{c}$
	Magnetic Stirrer	$48.80\pm2.47_{a}^{a}$	$41.35\pm1.74_{a}^{b}$	$44.15\pm2.27_{a}^{b}$	$36.60\pm0.70_{a}^{c}$
F. Religiosa	Orbital Shaker	$55.5\pm2.75_{a}^{a}$	$48.75\pm2.05_{a}^{c}$	$50.95\pm2.54_{a}^{b}$	$38.00\pm1.18_{a}^{d}$
	Sonication	$53.85\pm2.68_{b}^{a}$	$47.50\pm2.00_{b}^{c}$	$50.95\pm2.54_{b}^{b}$	$36.15\pm1.10_{b}^{d}$
	Magnetic Stirrer	$55.00\pm2.73_{a}^{a}$	$48.70\pm2.05_{a}^{c}$	$52.40\pm2.66_{a}^{b}$	$37.95\pm1.17_{a}^{d}$
F. retusa	Orbital Shaker	$46.30\pm2.78_{a}^{a}$	$36.85\pm1.55_{a}^{c}$	$42.65\pm2.63_{a}^{b}$	$23.40\pm0.56_{a}^{d}$
	Sonication	$45.20\pm2.74_{c}^{a}$	$35.65\pm1.50_{c}^{c}$	$40.60\pm2.55_{c}^{b}$	$20.50\pm0.44_{c}^{d}$
	Magnetic Stirrer	$45.95\pm2.77_{b}^{a}$	$36.80\pm1.55_{b}^{c}$	$41.50\pm2.63_{b}^{b}$	$21.52\pm0.48_{b}^{d}$

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

The results calculated for the DPPH radical scavenging potential of the extracts obtained from the fruit samples of selected species of *Ficus* were given in table 4.10 and these confirm that all the fruit samples studied follow the general trends of activity as observed for other antioxidant tests performed. The results for the DPPH radical scavenging activity of different extracts of fruits proved that the extraction solvents employed in this work had considerable effect on the radical scavenging and antioxidant activity of the samples tested. Among the different extracts obtained from the fruits of *Ficus* species, the IC_{50} value varied from 46.3±2.78 µg/mL to 20.5±0.44 µg/mL for the fruit of *F. retusa* and IC_{50} values of all the extracts obtained from the fruits of *F. retusa* by the application of different solvents and techniques were significantly ($p<0.05$) different from each other. IC_{50} value varied from 36.4±2.37 µg/mL to 20.6±0.53 µg/mL for the fruit of *F. bengalensis* and 55.5±2.75 µg/mL to 37.95±1.17 µg/mL for the fruit of *F. religiosa*. Significantly ($p<0.05$) different IC_{50} values were calculated for the fruit extracts of these two species obtained by the application of different solvents applied in the present study but the IC_{50} values calculated for these two samples under the influence of orbital shaker and magnetic stirrer were not significantly ($p<0.05$) different from each other. IC_{50} values varied from 49.7±2.51 µg/mL to 32.3±0.94 µg/mL for the fruit of *F. racemosa* under the influence of different solvents and techniques applied but there was no significant ($p<0.05$) difference among the values obtained under the influence of different techniques (orbital shaker, sonication, magnetic stirrer) for the same solvent. Similarly the values obtained by the application of 80% ethanol and 100% methanol were not significantly ($p<0.05$) different from each other. Variation in the IC_{50} values for the fruit sample of *F. infectoria* was from 57.25±2.82 µg/mL to 47.15±2.06 µg/mL and all the IC_{50} values obtained by the application of different solvents and techniques were significantly ($p<0.05$) different from each other . In all the fruit samples, the highest IC_{50} values (lowest antioxidant activity) were obtained for the extracts produced by the combination of 100% ethanol as solvent and orbital shaker as extraction

Table 4.11. Effect of extraction procedure on the DPPH radical scavenging activity (IC_{50} µg/mL) of the extracts obtained from the leaves of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$87.35 \pm 3.67_a^a$	$79.75 \pm 3.35_a^c$	$86.2 \pm 3.62_a^b$	$76.85 \pm 3.23_a^d$
	Sonication	$87.05 \pm 3.42_b^a$	$79.1 \pm 3.32_b^c$	$85.85 \pm 3.61_b^b$	$76.10 \pm 3.20_b^d$
	Magnetic Stirrer	$87.20 \pm 3.54_a^a$	$79.66 \pm 3.35_a^c$	$86.05 \pm 3.61_a^b$	$76.79 \pm 3.22_a^d$
F. infectoria	Orbital Shaker	$67.35 \pm 2.83_a^a$	$65.6 \pm 2.76_a^c$	$66.45 \pm 2.79_a^b$	$55.45 \pm 2.33_a^d$
	Sonication	$66.9 \pm 2.69_b^a$	$64.8 \pm 2.72_b^c$	$65.7 \pm 2.75_b^b$	$54.00 \pm 2.27_b^d$
	Magnetic Stirrer	$67.2 \pm 2.82_a^a$	$65.45 \pm 2.75_a^c$	$66.3 \pm 2.78_a^b$	$55.3 \pm 2.32_a^d$
F. Racemosa	Orbital Shaker	$76.55 \pm 3.22_a^a$	$65.85 \pm 2.77_a^c$	$71.75 \pm 3.01_a^b$	$55.10 \pm 2.31_a^d$
	Sonication	$75.9 \pm 3.19_a^a$	$64.85 \pm 2.72_a^c$	$71.20 \pm 2.99_a^b$	$50.40 \pm 2.12_a^d$
	Magnetic Stirrer	$76.47 \pm 3.20_a^a$	$65.69 \pm 2.76_a^c$	$71.70 \pm 3.01_a^b$	$53.40 \pm 2.24_a^d$
F. Religiosa	Orbital Shaker	$92.25 \pm 3.76_a^a$	$81.15 \pm 3.41_a^b$	$88.95 \pm 3.87_a^a$	$78.25 \pm 3.29_a^c$
	Sonication	$89.25 \pm 3.75_a^a$	$80.80 \pm 3.39_a^b$	$88.55 \pm 3.72_a^a$	$77.60 \pm 3.26_a^c$
	Magnetic Stirrer	$89.50 \pm 3.76_a^a$	$81.10 \pm 3.41_a^b$	$89.20 \pm 3.75_a^a$	$78.25 \pm 3.29_a^c$
F. retusa	Orbital Shaker	$48.20 \pm 2.02_a^a$	$36.90 \pm 1.63_a^b$	$37.40 \pm 1.57_a^b$	$33.10 \pm 1.39_a^c$
	Sonication	$46.50 \pm 1.95_b^a$	$34.80 \pm 1.57_b^b$	$36.95 \pm 1.5_b^b$	$31.15 \pm 1.31_b^c$

	Magnetic Stirrer	$47.65±2.00_a^a$	$36.85±1.63_a^b$	$37.00±1.55_a^b$	$32.75±1.38_a^c$

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

fruit extracts of *F. retusa*,fruits of *F. racemosa* , fruit samples of *F. religiosa* and then *F. infectoria.*

The extracts obtained from the leaf samples of selected species of *Ficus* by using different solvents and techniques also offered fairly good DPPH radical scavenging activity in terms of IC_{50} values (table 4.11). The leaf extracts produced by the combination of different solvents and techniques employed in this study also showed similar trends for DPPH scavenging potential as were appraised above for the fruits of these species. Briefly, in the same fashion, the leaf extracts produced by using 80%methanol and sonication offered the highest radical scavenging potential whereas those produced by 100% ethanol and shaker exhibited the least radical scavenging activity. Radical scavenging activity (IC_{50} values) of the leaf samples varied under the influence of different combinations of solvents with techniques applied for extraction. IC_{50} value ranged from 87.35±3.67 to 76.10±3.20 for *F. bengalensis,* 67.35±2.83 to 54.00±2.27 for *F. infectoria*, 76.55±3.22 to 50.40±2.12 for *F. racemosa*, 92.25±3.76 to 77.60±3.26 for *F. religiosa* and 48.20±2.02 to 31.15±1.31 for *F. retusa*. All the higher and lower IC_{50} values mentioned above for various leaf samples were significantly different from one another. The extracts obtained from the leaf samples of *F. bengalensis, F. infectoria* and *F. racemosa* exhibited significantly ($p<0.05$) different DPPH radical scavenging activity with respect to solvents applied for extraction but the IC_{50} values obtained for the leaves of *F. religiosa* and *F. retusa* by the application of absolute ethanol and absolute methanol were not significantly ($p<0.05$) different. DPPH radical scavenging activities were not significantly ($p<0.05$) different under the influence of orbital shaker and magnetic stirrer for the leaves of *F. bengalensis, F. infectoria* and *F. retusa* although DPPH radical scavenging activities for these plants were significantly ($p<0.05$) different when investigated under the influence of sonication from the activities calculated by the application of orbital shaker and magnetic stirrer. There was no significant ($p<0.05$) difference among the IC_{50} values calculated by the application of different extraction techniques for the leaves of *F. racemosa* and *F. religiosa.* The leaf sample of *F.religiosa* offered the lowest radical scavenging activity among all the leaf samples of *Ficus* species analyzed in the present study with all the combinations of solvent and technique while the highest radical scavenging activity was exhibited by the leaf samples of *F. retusa*.

Table 4.12. Effect of extraction procedure on the DPPH radical scavenging activity (IC_{50} µg/mL) of the extracts obtained from the bark of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
F. bengalensis	Orbital Shaker	$70.65\pm2.97_{a}^{a}$	$53.70\pm2.26_{a}^{c}$	$64.80\pm2.72_{a}^{b}$	$39.25\pm1.65_{a}^{d}$
	Sonication	$69.90\pm2.94_{b}^{a}$	$52.80\pm2.22_{b}^{c}$	$63.95\pm2.69_{b}^{b}$	$37.85\pm1.59_{b}^{d}$
	Magnetic Stirrer	$70.56\pm2.97_{a}^{a}$	$53.45\pm2.24_{a}^{c}$	$64.70\pm2.72_{a}^{b}$	$38.90\pm1.63_{a}^{d}$
F. infectoria	Orbital Shaker	$71.55\pm3.01_{a}^{a}$	$62.05\pm2.61_{a}^{c}$	$70.55\pm2.96_{a}^{b}$	$57.15\pm2.40_{a}^{d}$
	Sonication	$70.70\pm2.97_{b}^{a}$	$61.00\pm2.56_{b}^{c}$	$69.05\pm2.90_{b}^{b}$	$56.45\pm2.37_{b}^{d}$
	Magnetic Stirrer	$71.40\pm3.00_{a}^{a}$	$62.05\pm2.74_{a}^{c}$	$70.35\pm2.86_{a}^{b}$	$57.05\pm2.41_{a}^{d}$
F. Racemosa	Orbital Shaker	$62.20\pm2.61_{a}^{a}$	$45.05\pm1.89_{a}^{c}$	$61.50\pm2.58_{a}^{b}$	$39.65\pm1.67_{a}^{d}$
	Sonication	$61.90\pm2.57_{b}^{a}$	$43.60\pm1.83_{b}^{c}$	$60.45\pm2.54_{b}^{b}$	$37.70\pm1.58_{b}^{d}$
	Magnetic Stirrer	$62.10\pm2.58_{a}^{a}$	$44.95\pm1.89_{a}^{c}$	$61.35\pm2.58_{a}^{b}$	$39.56\pm1.64_{a}^{d}$
F. Religiosa	Orbital Shaker	$70.5\pm2.96_{a}^{a}$	$57.30\pm2.41_{a}^{c}$	$68.95\pm2.89_{a}^{b}$	$49.65\pm2.09_{a}^{d}$
	Sonication	$69.55\pm2.92_{b}^{a}$	$56.05\pm2.35_{b}^{c}$	$68.05\pm2.86_{b}^{b}$	$48.21\pm2.02_{b}^{d}$
	Magnetic Stirrer	$70.20\pm2.95_{a}^{a}$	$57.15\pm2.40_{a}^{c}$	$68.91\pm2.89_{a}^{b}$	$49.60\pm2.08_{a}^{d}$
F. retusa	Orbital Shaker	$67.75\pm2.84_{a}^{a}$	$53.15\pm2.23_{a}^{c}$	$64.75\pm2.72_{a}^{b}$	$41.62\pm1.75_{a}^{d}$

	Sonication	$66.60\pm2.80_b^a$	$52.25\pm2.19_b^c$	$63.55\pm2.67_b^b$	$40.31\pm1.69_b^d$
	Magnetic Stirrer	$67.71\pm2.78_a^a$	$53.01\pm2.33_a^c$	$64.70\pm2.69_a^b$	$41.55\pm1.75_a^d$

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

DPPH radical scavenging capacity of extracts produced by different solvents and extraction techniques from the bark samples of selected species of *Ficus* in terms of IC_{50} values was given in table 4.12 and this reveals that effect of solvent and technique on the radical scavenging activity of the bark samples studied was in line to those observed previously from the fruits and leaves. According to the data, IC_{50} values improved significantly for the same sample when different combinations of solvent with technique were employed for extraction. Radical scavenging activity (IC_{50} value) improved from 70.65±2.97 to 37.85±1.59 for the bark extracts of *F. bengalensis*, 71.55±3.01 to 56.45±2.37 for *F. infectoria*, 62.20±2.61 to 37.70±1.58 for *F. racemosa*, 70.5±2.96 to 48.21±2.02 for *F. religiosa* and 67.75±2.84 to 40.31±1.69 for *F. retusa* by the application of different combinations of solvent with technique. Significantly ($p<0.05$) different radical scavenging activities obtained by the application of different solvents from all the plants tested in the present study and statistical ranking of solvents on the basis of their DPPH radical scavenging activity was 80% methanol> 80% ethanol>100% methanol> 100% ethanol. IC_{50} values obtained by the application of orbital shaker and magnetic stirrer from the barks of selected species of *Ficus* were not significantly ($p<0.05$) different from each other. These results also support that radical scavenging activity and hence the antioxidant activity of *Ficus* bark extracts is dependent upon the nature of extraction solvent as well as the mode of extraction technique. As far as the extent of radical scavenging capacity of different barks is concerned among the selected *Ficus* species, the bark extracts of *F. racemosa* and *F. infectoria* offered the highest and the lowest radical scavenging activity among others.

Tert-butyl-1- hydroxytoluene (BHT) was used as reference standard in the present study and its IC_{50} value was 33.88±0.06 µg/mL when we compare this value with that of samples investigated in the present study then it is clear that most of the samples have fairly good DPPH radical scavenging activity. IC_{50} values of the extracts obtained from the fruits of *F. bengalensis* and *F. retusa* by employing 80% methanol with sonication were significantly lower than that of BHT. The IC_{50} values of the extracts obtained from the *F. racemosa* fruits, *F. religiosa* fruits, *F. retusa* leaves, *F. bengalensis* bark and *F. racemosa* bark were comparable to the IC_{50} value of BHT while IC_{50} values of other samples were higher than that of BHT.

All the samples of fruit, leaf and bark from the selected species of *Ficus* offered fairly good radical scavenging activity and is comparable with the DPPH radical scavenging activity of syringaldehyde (IC_{50}= 74.1±0.7 µg/mL) reported by Ao *et al.*, (2008) and that of ascorbic acid (IC_{50}= .068 mg/mL) reported by Debib *et al.*, (2013). Ao *et al.*, (2008) also reported IC_{50} values for the fruit (7.3 µg/mL), leaf (21.4 µg/mL) and bark (7.3 µg/mL) samples of *F. microcarpa* which are lower than the IC_{50} values for fruit (20.5±0.44 µg/mL), leaf (31.15±1.31 µg/mL) and bark (40.3±1.69 µg/mL) investigated in the present study. Thingbaijam *et al.*, (2012) reported EC_{50} value equal to 251µg/mL for the leaf sample of *F.auriculata* and this value is much higher than all the IC_{50} value for the leaf samples of selected species of *Ficus* reported in present study. Reported IC_{50} value (74±2.39 µg/mL) by Abdel-Hamed *et al.*, (2009) for the leaf samples of *F. virens* (*F. infectoria*) using methanol as solvent is in close agreement with IC_{50} value 71.55±3.01 µg/mL determined in the present study for the same plant with same solvents. Manian *et al.*, (2008) reported IC_{50} value for the stem bark of *F. racemosa* equal to 21.5µg/mL and this value is lower than our calculated IC_{50} value 37.7±1.58 µg/mL for the same part of the same species. Reported IC_{50} value (0.208 mg/ml) for the fruit of *F. carica* by Verma *et al.*, (2010) is much higher than our calculated values for the fruit samples. Such variations of radical scavenging activity among samples of the same species of *Ficus* and within different species of *Ficus* might be in due part to the different genetic makeup of the species as well as agroclimatic and geographical variations of the regions of the harvest of these plants.

4.5. Influence of extraction process on reducing power

Reducing power of the extracts obtained from the samples was determined at five different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) of each extract by measuring the absorbance with colorimeter. The average of all the absorbance values noted for different concentrations of each sample was reported as the reducing power of that sample. Higher the averaged value of absorbance for a sample means higher reducing power of that sample and vice versa. The results for the reducing power of fruits, leaves and barks of selected species of *Ficus* are presented in table 4.13, 4.14 and 4.15, respectively.

The reducing power of the extracts obtained by the application of different solvent systems and techniques exhibited very good reducing potential. As is the case for other antioxidant tests performed, reducing power of the extracts obtained by using 80% methanol as solvent and sonication as extraction technique demonstrated the higher reducing potential as compared to the extracts obtained by the application of other solvents and techniques. In most of

n the reducing power (absorbance at 700 nm) of extracts obtained
Table 4.13. Effect of extraction procedure on the o from
the fruits of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$0.90\pm0.04_{c}^{d}$	$1.08\pm0.05_{c}^{b}$	$1.00\ \pm0.04_{c}^{c}$	$1.53\pm0.06_{c}^{a}$
	Sonication	$0.93\pm0.04_{a}^{d}$	$1.13\pm0.05_{a}^{b}$	$1.06\pm0.04_{a}^{c}$	$1.57\pm0.07_{a}^{a}$
	Magnetic Stirrer	$0.91\pm0.04_{b}^{d}$	$1.10\pm0.05_{b}^{b}$	$1.04\pm0.04_{b}^{c}$	$1.55\pm0.06_{b}^{a}$
F. infectoria	Orbital Shaker	$0.85\pm0.04_{a}^{b}$	$0.79\pm0.03_{a}^{b}$	$0.82\pm0.03_{a}^{b}$	$0.96\pm0.04_{a}^{a}$
	Sonication	$0.74\pm0.03_{a}^{b}$	$0.86\pm0.04_{a}^{b}$	$0.87\pm0.04_{a}^{b}$	$1.02\pm0.04_{a}^{a}$
	Magnetic Stirrer	$0.71\pm0.03_{a}^{b}$	$0.80\pm0.03_{a}^{b}$	$0.83\pm0.03_{a}^{b}$	$0.97\pm0.04_{a}^{a}$
F. Racemosa	Orbital Shaker	$0.84\pm0.04_{b}^{d}$	$1.12\pm0.05_{b}^{b}$	$0.92\pm0.04_{b}^{c}$	$1.33\pm0.06_{b}^{a}$
	Sonication	$0.91\pm0.04_{a}^{d}$	$1.26\pm0.05_{a}^{b}$	$0.95\pm0.04_{a}^{c}$	$1.48\pm0.06_{a}^{a}$
	Magnetic Stirrer	$0.85\pm0.04_{b}^{d}$	$1.20\pm0.05_{b}^{b}$	$0.93\pm0.04_{b}^{c}$	$1.39\pm0.06_{b}^{a}$
F. Religiosa	Orbital Shaker	$0.69\pm0.03_{c}^{d}$	$1.02\pm0.04_{c}^{b}$	$0.74\pm0.03_{c}^{c}$	$1.31\pm0.05_{c}^{a}$
	Sonication	$0.73\pm0.03_{a}^{d}$	$1.06\pm0.04_{a}^{b}$	$0.78\pm0.03_{a}^{c}$	$1.37\pm0.06_{a}^{a}$
	Magnetic Stirrer	$0.71\pm0.03_{b}^{d}$	$1.04\pm0.04_{b}^{b}$	$0.75\pm0.03_{b}^{c}$	$1.35\pm0.06_{b}^{a}$
F. retusa	Orbital Shaker	$0.83\pm0.03_{a}^{c}$	$1.27\pm0.05_{a}^{b}$	$0.87\pm0.04_{a}^{c}$	$1.77\pm0.07_{a}^{a}$
	Sonication	$0.85\pm0.04_{a}^{c}$	$1.26\pm0.05_{a}^{b}$	$0.93\pm0.04_{a}^{c}$	$1.93\pm0.08_{a}^{a}$
	Magnetic Stirrer	$0.84\pm0.04_{a}^{c}$	$1.29\pm0.05_{a}^{b}$	$0.90\pm0.04_{a}^{c}$	$1.84\pm0.08_{a}^{a}$

n the reducing power (absorbance at 700 nm) of extracts obtained

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

the cases the order of efficiency of the solvents used in the present study towards extraction of potent reducing agents form *Ficus* species was found to be 80% methanol> 80% ethanol> 100% methanol> 100% ethanol whereas with regard to efficiency of techniques this order was sonication extraction> magnetic stirring> orbital shaker extraction. Zahid *et al*., (2012) conducted a study to evaluate the effect of solvent on the antioxidant and antimicrobial activity of different parts of *P. pinnata* (L.) Pierre and recorded similar effect of solvents on the reducing power of the extracts as observed in the present study. The present reducing power trends exhibited by different solvent extracts of *Ficus* species analyzed were also in close resemblance to those reported by Sultana *et al.*,(2009) and Zahid *et al*., (2012) for the similar solvent extracts derived from different botanical sources.

Different solvents and techniques used in the present study have significant effect on the reducing potential of the extracts obtained from the fruits of selected species of *Ficus*. The reducing potential, in terms of absorbance data, of the fruits of *F. retusa* varied from 0.83±0.03 to 1.93±0.08 while that of fruits of *F. religiosa* 0.69±0.03 to 1.37±0.06 as function of most efficient extraction combination of 80% methanol and sonication extraction. The reducing potentials of the extracts acquired from the fruit samples of *F. racemosa*, *F. benglensis* and *F. infectoria* ranged from 0.84±0.04 to 1.48±0.06, 0.9±0.04 to 1.57±0.07 and 0.71±0.03 to 1.02±0.04, respectively. Although there is fair enough variation from lower to higher values mentioned above but the statistical analysis revealed that there was no significant ($p<0.05$) variation obtained by the application of different techniques (orbital shaker, sonication, magnetic stirrer) for the fruit samples of *F. infectoria* and *F. retusa*. But in case of fruit samples of *F. benglensis* and *F. religiosa* significantly ($p<0.05$) different values of reducing power were obtained by the application of extraction techniques used in the present study. Fruit samples of *F. benglensis, F. racemosa* and *F. religiosa* exhibited significantly ($p<0.05$) different reducing power when different solvents (80% ethanol, 80% methanol, 100% ethanol and 100% methanol) were applied. Reducing power of the fruits of *F. retusa* were not significantly ($p<0.05$) different when obtained under the influence of absolute methanol and absolute ethanol. No significant ($p<0.05$) variation was observed in the reducing power of the extracts obtained from the fruits of *F. infectoria* by the application of 80% ethanol, 100% ethanol and 100% methanol. Comparison of reducing potential of fruit extracts of selected species of *Ficus* revealed that extracts obtained from the fruits of *F. retusa* have the highest reducing potential when the

n the reducing power (absorbance at 700 nm) of extracts obtained
Table 4.14. Effect of extraction procedure on the o from
the leaves of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$0.28\pm0.01_{c}^{d}$	$0.44\pm0.02_{c}^{b}$	$0.30\pm0.01_{c}^{c}$	$0.50\pm0.02_{c}^{a}$
	Sonication	$0.30\pm0.01_{a}^{d}$	$0.48\pm0.02_{a}^{b}$	$0.33\pm0.01_{a}^{c}$	$0.54\pm0.02_{a}^{a}$
	Magnetic Stirrer	$0.29\pm0.01_{b}^{d}$	$0.47\pm0.02_{b}^{b}$	$0.32\pm0.01_{b}^{c}$	$0.53\pm0.02_{b}^{a}$
F. infectoria	Orbital Shaker	$0.59\pm0.03_{c}^{c}$	$0.64\pm0.03_{c}^{b}$	$0.62\pm0.03_{c}^{b}$	$0.88\pm0.04_{c}^{a}$
	Sonication	$0.66\pm0.03_{a}^{c}$	$0.71\pm0.03_{a}^{b}$	$0.68\pm0.03_{a}^{b}$	$0.91\pm0.04_{a}^{a}$
	Magnetic Stirrer	$0.62\pm0.03_{b}^{c}$	$0.66\pm0.03_{b}^{b}$	$0.66\pm0.03_{b}^{b}$	$0.89\pm0.04_{b}^{a}$
F. Racemosa	Orbital Shaker	$0.45\pm0.02_{b}^{d}$	$0.69\pm0.03_{b}^{b}$	$0.55\pm0.02_{b}^{c}$	$0.85\pm0.04_{b}^{a}$
	Sonication	$0.48\pm0.02_{a}^{d}$	$0.74\pm0.03_{a}^{b}$	$0.58\pm0.02_{a}^{c}$	$0.95\pm0.04_{a}^{a}$
	Magnetic Stirrer	$0.47\pm0.02_{ab}^{d}$	$0.70\pm0.03_{ab}^{b}$	$0.57\pm0.02_{ab}^{c}$	$0.89\pm0.04_{ab}^{a}$
F. Religiosa	Orbital Shaker	$0.21\pm0.01_{b}^{c}$	$0.31\pm0.01_{b}^{b}$	$0.23\pm0.01_{b}^{c}$	$0.40\pm0.02_{b}^{a}$
	Sonication	$0.23\pm0.01_{a}^{c}$	$0.35\pm0.01_{a}^{b}$	$0.24\pm0.01_{a}^{c}$	$0.44\pm0.02_{a}^{a}$
	Magnetic Stirrer	$0.22\pm0.01_{ab}^{c}$	$0.34\pm0.01_{ab}^{b}$	$0.23\pm0.01_{ab}^{c}$	$0.41\pm0.02_{ab}^{a}$
F. retusa	Orbital Shaker	$0.88\pm0.04_{c}^{d}$	$1.09\pm0.05_{c}^{b}$	$1.01\pm0.04_{c}^{c}$	$1.16\pm0.05_{c}^{a}$
	Sonication	$0.91\pm0.04_{a}^{d}$	$1.14\pm0.05_{a}^{b}$	$1.07\pm0.04_{a}^{c}$	$1.21\pm0.05_{a}^{a}$
	Magnetic Stirrer	$0.89\pm0.04_{b}^{d}$	$1.11\pm0.05_{b}^{b}$	$1.04\pm0.04_{b}^{c}$	$1.17\pm0.05_{b}^{a}$

n the reducing power (absorbance at 700 nm) of extracts obtained

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

solvent was 80% methanol or 80% ethanol while the extracts from the fruits of *F. bengalensis* exhibited the highest reducing potential when the solvent was 100% methanol or 100% ethanol in combination with all the techniques used in the present research. On the other hand, extracts obtained from the fruits of *F. infectoria* showed the lowest reducing potential among the fruit samples when the extraction solvent was 80% methanol or 80% ethanol and the extracts obtained from the fruits of *F. religiosa* exhibited the lowest reducing power among the fruit samples when absolute methanol or absolute ethanol was used as extraction solvent.

Different combinations of extraction solvents and techniques also have significant effect on the reducing potential of the leaf samples of *Ficus* species analyzed in the present study. Significant improvement was observed in the reducing power of the leaves when different combination of solvent with technique was employed for extraction. The reducing power of the leaves of *F. bengalensis* improved from 0.28±0.01 to 0.54±0.02 under the influence of different solvents and technique applied for extraction. Improvement in the reducing power was from 0.59±0.03 to 0.91±0.04 for the leaves of *F. infectoria*, from 0.45±0.02 to 0.95±0.04 for the leaves of *F. racemosa*, from 0.21±0.01 to 0.44±0.02 for the leaves of *F. religiosa* and from 0.88±0.04 to for the leaves of 1.21±0.05 for the leaves of *F. retusa*. The reducing power of the leaf extracts, produced by different solvents and techniques, also exhibited trends in line to those of fruit extracts using the same extraction solvents and techniques. Extracts obtained by the application of 80% methanol and sonication had the highest reducing power while those obtained by 100% ethanol using orbital shaker showed the least reducing power. In case of leaf samples of *F. religiosa*, extracts obtained by the application of 100% ethanol and 100% methanol did not show significantly ($p<0.05$) different reducing power and in case of *F. infectoria*, the reducing power of the extracts obtained by the application of 80% ethanol and 100% methanol was not significantly ($p<0.05$) different. For the leaf samples of *F. bengalensis*, *F. racemosa* and *F. retusa*, the effect of different solvents on the reducing power was significant ($p<0.05$). The effect of all the extraction techniques used in the present study on the reducing power of the leaf samples was significant ($p<0.05$). Among leaves of the *Ficus* species selected, the extracts from the leaves of *F. retusa* exhibited the highest reducing potential while the extracts obtained from the leaf samples of *F.religiosa* showed the lowest reducing potential.

n the reducing power (absorbance at 700 nm) of extracts obtained
Table 4.15. Effect of extraction procedure on the o from
the bark of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$0.59\pm0.02_{c}^{d}$	$0.86\pm0.04_{c}^{b}$	$0.68\pm0.03_{c}^{c}$	$1.13\pm0.05_{c}^{a}$
	Sonication	$0.61\pm0.03_{a}^{d}$	$0.89\pm0.04_{a}^{b}$	$0.71\pm0.03_{a}^{c}$	$1.16\pm0.05_{a}^{a}$
	Magnetic Stirrer	$0.61\pm0.02_{b}^{d}$	$0.88\pm0.04_{b}^{b}$	$0.69\pm0.03_{b}^{c}$	$1.14\pm0.05_{b}^{a}$
F. infectoria	Orbital Shaker	$0.54\pm0.02_{b}^{d}$	$0.72\pm0.03_{b}^{b}$	$0.60\pm0.03_{b}^{c}$	$0.83\pm0.03_{b}^{a}$
	Sonication	$0.61\pm0.03_{a}^{d}$	$0.75\pm0.03_{a}^{b}$	$0.64\pm0.03_{a}^{c}$	$0.85\pm0.04_{a}^{a}$
	Magnetic Stirrer	$0.59\pm0.02_{ab}^{d}$	$0.73\pm0.03_{ab}^{b}$	$0.61\pm0.03_{ab}^{c}$	$0.84\pm0.04_{ab}^{a}$
F. Racemosa	Orbital Shaker	$0.76\pm0.03_{c}^{d}$	$1.03\pm0.04_{c}^{b}$	$0.78\pm0.03_{c}^{c}$	$1.14\pm0.05_{c}^{a}$
	Sonication	$0.79\pm0.03_{a}^{d}$	$1.08\pm0.05_{a}^{b}$	$0.81\pm0.03_{a}^{c}$	$1.19\pm0.05_{a}^{a}$
	Magnetic Stirrer	$0.77\pm0.03_{b}^{d}$	$1.05\pm0.04_{b}^{b}$	$0.79\pm0.03_{b}^{c}$	$1.16\pm0.05_{b}^{a}$
F. Religiosa	Orbital Shaker	$0.61\pm0.03_{c}^{d}$	$0.84\pm0.04_{c}^{b}$	$0.63\pm0.03_{c}^{c}$	$0.99\pm0.04_{c}^{a}$
	Sonication	$0.63\pm0.03_{a}^{d}$	$0.86\pm0.04_{a}^{b}$	$0.67\pm0.03_{a}^{c}$	$1.02\pm0.04_{a}^{a}$
	Magnetic Stirrer	$0.62\pm0.03_{b}^{d}$	$0.84\pm0.04_{b}^{b}$	$0.65\pm0.03_{b}^{c}$	$1.00\pm0.04_{b}^{a}$
F. retusa	Orbital Shaker	$0.62\pm0.03_{c}^{d}$	$0.89\pm0.04_{c}^{b}$	$0.71\pm0.03_{c}^{c}$	$1.10\pm0.05_{c}^{a}$
	Sonication	$0.67\pm0.03_{a}^{d}$	$0.92\pm0.04_{a}^{b}$	$0.73\pm0.03_{a}^{c}$	$1.14\pm0.05_{a}^{a}$
	Magnetic Stirrer	$0.64\pm0.03_{b}^{d}$	$0.90\pm0.04_{b}^{b}$	$0.71\pm0.02_{b}^{c}$	$1.12\pm0.05_{b}^{a}$

n the reducing power (absorbance at 700 nm) of extracts obtained

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

In relation to extraction solvent and techniques, the extracts obtained from the bark samples of *Ficus* species investigated in the present study also exhibited reducing potential in the same fashion as was recorded for the other parts of the species selected. In most of the cases, the reducing power of the bark extracts, obtained by the application of 80% methanol as extraction solvent and sonication as extraction technique, were higher among others indicating this extraction system to be most efficient. Significantly ($p<0.05$) different reducing power was exhibited by the bark samples of all the tested species under the influence of different solvents and techniques applied for extraction in the present study. The statistical ranking of solvents on the basis of the reducing power of extracts obtained by their application was 80% methanol > 80% ethanol > 100% methanol > 100% ethanol and the ranking for techniques was sonication > magnetic stirrer > orbital shaker. Reducing power of the bark samples varied over a wide range when we move from the least efficient combination (ethanol with orbital shaker) to the most efficient combination (methanol with sonication). Reducing power of the bark samples ranged from 0.59±0.02 to 1.16±0.05 for *F. bengalensis*, 0.54±0.02 to 0.85±0.04 for *F. infectoria*, 0.76±0.03 to 1.19±0.05 for *F. racemosa*, 0.61±0.03 to 1.02±0.04 for *F. religiosa* and 0.62±0.03 to 1.14±0.05 for *F. retusa*. All the lower values were significantly ($p<0.05$) different from their respective higher values. Within the *Ficus* species selected, the extracts from the bark of *F. racemosa* showed the highest reducing potential while the extracts from the bark of *F. infectoria* exhibited the lowest reducing potential regardless of the choice of solvent and technique employed.

Tert-butyl-1- hydroxytoluene (BHT) was used as reference standard in the present study and its reducing power was equal to 1.64±0.02 which was lower than the reducing power of the extracts obtained from the fruits of *F. retusa* by the application of 80% methanol with all the three techniques. Reducing power of the extracts obtained from the fruits of *F. bengalensis* by employing 80% methanol was comparable to the reducing power of BHT while other samples investigated in the present study exhibited lower reducing power than that of BHT.

The reducing potential of different parts of the selected species of *Ficus* assessed in the present work can be supported by some previous researchers who also reported that different parts of these plants are potential source of phytoextracts with potent reducing potential and antioxidant activity. Melinda *et al.*, (2010) reported reducing power of leaves extract (2mg/mL concentration) of *F. religiosa* equal to 0.788±0.02. Sultana *et al.*, (2009) used 10mg/mL concentration of the extracts from fruit of *F. religiosa* produced by four solvents (80% methanol,

80% ethanol, 100% methanol and 100% ethanol) and assessed their its reducing power to be 3.77 ±0.10 (80% methanol) , 2.03 ± 0.06 (80% ethanol), 2.16 ± 0.08 (100% methanol) and 1.28 ± 0.04 (100% ethanol). Although we cannot compare these reported values exactly with those determined in the present study because of the concentration variations but it could be guessed that the effect of solvent is almost similar to that we recorded. Shi *et al.*, (2011) evaluated reducing potential of the methanolic extracts obtained from the leaves of *F. racemosa* and *F. virens* at different concentrations of 0.05, 0.1, 0.15, 0.2 and 0.25 mg/mL and concluded that reducing power increased by increasing the concentration. The reported reducing potential at 0.25 mg/mL concentration level was 0.26±0.001 for *F. racemosa* and 0.66±0.021 for *F. virens*. Anandjiwala *et al.,* (2008) investigated the reducing potential of methanolic extract from the barks of *F. bengalensis*, *F. racemosa*, *F. religiosa* and *F. virens* at concentration levels of 50, 100, 150, 200 and 300 μg/mL. The reducing potential investigated at 300 μg/mL were 1.0154, 1.018, .0946 and 0.766 for the barks of *F. bengalensis, F. racemosa, F. religiosa* and *F. virens*, respectively. Although comparison is not possible between the published reports and our observations for reducing potential of *Ficus* species but it is clear that extracts obtained from the different parts of *Ficus* plants have fairly good reducing potential.

4.6. Inhibition of peroxidation in linoleic acid

The extracts from fruit, leaf and bark samples of the selected species of *Ficus* were investigated for their ability to inhibit peroxidation of linoleic acid. The results obtained revealed that these samples have fair good potential to inhibit the linoleic acid peroxidation. In most of the food samples lipids are oxidized and peroxides are produced which further decomposes into aldehydes or ketones and/or alcohols and these secondary oxidation products create smell and spoil the food. Evaluation of lipid per oxidation inhibition potential of plant extracts is an important indicator for exploring their antioxidant principles and applications. The results obtained for this antioxidant test are represented as %age inhibition and are tabulated in table 4.16 for fruits, in table 4.17 for leaves and in table 4.18 for barks of selected species of *Ficus*. Different combinations of solvent with technique used in the present study affected the inhibitory potential of the *Ficus* samples in the similar manner as recorded previously in case of DPPH

Table 4.16. Effect of extraction procedure on the % age inhibition of peroxidation in linoleic acid by the extracts obtained from the fruits of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$60.32\pm2.84_{b}^{d}$	$65.52\pm3.08_{b}^{c}$	$61.75\pm2.9_{b}^{b}$	$70.31\pm3.3_{b}^{a}$
	Sonication	$61.06\pm2.87_{a}^{d}$	$67.55\pm3.17_{a}^{c}$	$63.98\pm2.98_{a}^{b}$	$72.48\pm3.41_{a}^{a}$
	Magnetic Stirrer	$61.00\pm2.87_{ab}^{d}$	$66.27\pm3.11_{ab}^{c}$	$65.49\pm3.08_{ab}^{b}$	$71.10\pm3.34_{ab}^{a}$
F. infectoria	Orbital Shaker	$44.95\pm2.11_{b}^{c}$	$48.38\pm2.27_{b}^{b}$	$47.81\pm2.25_{b}^{b}$	$51.91\pm2.44_{b}^{a}$
	Sonication	$45.69\pm2.15_{a}^{c}$	$50.83\pm2.39_{a}^{b}$	$50.23\pm2.36_{a}^{b}$	$54.54\pm2.56_{a}^{a}$
	Magnetic Stirrer	$45.55\pm2.14_{b}^{c}$	$48.63\pm2.19_{b}^{b}$	$48.06\pm2.26_{b}^{b}$	$52.18\pm2.45_{b}^{a}$
F. Racemosa	Orbital Shaker	$59.64\pm2.80_{b}^{c}$	$65.90\pm3.10_{b}^{b}$	$65.12\pm3.06_{b}^{b}$	$70.70\pm3.32_{b}^{a}$
	Sonication	$64.69\pm3.04_{a}^{c}$	$67.93\pm3.19_{a}^{b}$	$65.13\pm3.06_{a}^{b}$	$72.89\pm3.43_{a}^{a}$
	Magnetic Stirrer	$61.42\pm2.89_{ab}^{c}$	$66.64\pm3.13_{ab}^{b}$	$65.86\pm3.10_{ab}^{b}$	$71.51\pm3.36_{ab}^{a}$
F. Religiosa	Orbital Shaker	$59.27\pm2.79_{b}^{d}$	$63.21\pm2.97_{b}^{b}$	$61.53\pm2.89_{b}^{c}$	$66.8\pm3.14_{b}^{a}$
	Sonication	$60.31\pm2.83_{a}^{d}$	$64.19\pm3.02_{a}^{b}$	$63.43\pm2.98_{a}^{c}$	$68.87\pm3.24_{a}^{a}$
	Magnetic Stirrer	$59.83\pm2.81_{b}^{d}$	$62.97\pm2.96_{b}^{b}$	$62.22\pm2.92_{b}^{c}$	$67.56\pm3.18_{b}^{a}$
F. retusa	Orbital Shaker	$63.08\pm2.96_{c}^{d}$	$71.52\pm3.36_{c}^{b}$	$66.97\pm3.15_{c}^{c}$	$76.74\pm3.61_{c}^{a}$
	Sonication	$65.38\pm3.07_{a}^{d}$	$73.77\pm3.47_{a}^{b}$	$70.86\pm3.33_{a}^{c}$	$79.11\pm3.72_{a}^{a}$
	Magnetic Stirrer	$63.76\pm3.00_{b}^{d}$	$72.33\pm3.40_{b}^{b}$	$69.47\pm3.27_{b}^{c}$	$77.61\pm3.65_{b}^{a}$

age inhibition of peroxidation in linoleic acid by the extracts

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

radical scavenging and reducing power assays. The extracts, produced from different parts of *Ficus* species selected, by applying 80% methanol as solvent and sonication as extraction technique were the most efficient towards inhibition of linoleic acid peroxidation. In this context, the order of efficiency of the extraction solvents was 80%methanol> 80% ethanol> 100% methanol> 100% ethanol if the extraction technique remains the same. Similarly, the order of efficiency of techniques was sonication extraction> magnetic stirring> orbital shaking, if the extraction solvent remains the same. The most efficient combination of technique with solvent was sonication with 80% methanol and the least efficient combination was 100% ethanol with orbital shaking.

Inhibition of linoleic acid peroxidation by the extracts obtained from the fruits of selected species of *Ficus* varied noticeably when the solvent and technique used for extraction was changed. As function of extraction solvent and techniques employed, the variations in the percentage inhibition of peroxidation for the fruits of *F. retusa*, *F. racemosa*, *F. infectoria* , *F. benglensis* and *F. religiosa* were 63.08±2.96 to 79.11±3.72, 59.64±2.8% to 72.89±3.43, 44.95±2.11 to 54.54±2.56, 60.32±2.84 to 72.48±3.41 and 59.27±2.79 to 68.87±3.24, respectively. Significantly ($p<0.05$) different percentage inhibitions were observed under the influence of different solvents applied for extraction from the fruit samples of *F. benglensis*, *F. religiosa* and *F. retusa* but there was no significant difference observed between the %age inhibitions when 80% ethanol and 100% methanol was applied on the fruit samples of *F. infectoria* and *F. racemosa*. The extracts obtained from the fruit samples of *F. bengalensis*, *F. racemosa* and *F. retusa* by the application of different extraction techniques showed significantly ($p<0.05$) different %age inhibition of linoleic acid but the extracts obtained from the fruits of *F. infectoria* and *F. religiosa* by the application of orbital shaker and magnetic stirrer did not show any significant ($p<0.05$) difference in their ability to inhibit linoleic acid. Overall, the extracts obtained from the fruits of *F. retusa* by applying all the combinations of solvent and technique were proved to be the most efficient among all the tested fruit samples for their ability to inhibit peroxidation in linoleic acid. On the other hand, the extracts obtained from the fruits of *F. infectoria* by applying all the combinations of solvent and technique were proved to be the least efficient among all the tested fruit samples for their ability to inhibit peroxidation in linoleic acid.

Table 4.17. Effect of extraction procedure on the % age inhibition of peroxidation in linoleic acid by the extracts obtained from the leaves of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$33.09\pm1.56_{b}^{c}$	$35.74\pm1.68_{b}^{b}$	$34.93\pm1.64_{b}^{bc}$	$38.35\pm1.80_{b}^{a}$
	Sonication	$35.89\pm1.69_{a}^{c}$	$36.56\pm1.72_{a}^{b}$	$36.00\pm1.69_{a}^{bc}$	$39.23\pm1.84_{a}^{a}$
	Magnetic Stirrer	$35.09\pm1.65_{ab}^{c}$	$35.74\pm1.68_{ab}^{b}$	$35.32\pm1.66_{ab}^{bc}$	$38.35\pm1.80_{ab}^{a}$
F. infectoria	Orbital Shaker	$43.60\pm2.05_{b}^{d}$	$44.41\pm2.09_{b}^{b}$	$43.89\pm2.06_{b}^{c}$	$47.66\pm2.24_{b}^{a}$
	Sonication	$44.96\pm2.11_{a}^{d}$	$45.80\pm2.15_{a}^{b}$	$45.79\pm2.15_{a}^{c}$	$49.14\pm1.99_{a}^{a}$
	Magnetic Stirrer	$43.73\pm2.06_{b}^{d}$	$44.54\pm2.09_{b}^{b}$	$44.01\pm2.07_{b}^{c}$	$47.79\pm2.25_{b}^{a}$
F. Racemosa	Orbital Shaker	$42.00\pm1.97_{c}^{b}$	$42.78\pm2.01_{c}^{b}$	$42.27\pm1.99_{c}^{b}$	$45.90\pm2.16_{c}^{a}$
	Sonication	$47.62\pm2.24_{a}^{b}$	$48.50\pm2.28_{a}^{b}$	$44.93\pm2.11_{a}^{b}$	$52.04\pm2.45_{a}^{a}$
	Magnetic Stirrer	$44.04\pm2.07_{b}^{b}$	$44.85\pm2.11_{b}^{b}$	$44.33\pm2.08_{b}^{b}$	$48.13\pm2.26_{b}^{a}$
F. Religiosa	Orbital Shaker	$28.41\pm1.34_{b}^{bc}$	$29.15\pm1.41_{b}^{b}$	$27.00\pm1.27_{b}^{c}$	$31.94\pm1.51_{b}^{a}$
	Sonication	$30.03\pm1.41_{a}^{bc}$	$30.58\pm1.44_{a}^{b}$	$29.01\pm1.36_{a}^{c}$	$32.82\pm1.54_{a}^{a}$
	Magnetic Stirrer	$29.41\pm1.38_{a}^{bc}$	$29.95\pm1.41_{a}^{b}$	$29.60\pm1.39_{a}^{c}$	$32.14\pm1.51_{a}^{a}$
F. retusa	Orbital Shaker	$53.45\pm2.51_{b}^{c}$	$57.92\pm2.72_{b}^{b}$	$57.87\pm2.72_{b}^{b}$	$62.84\pm2.95_{b}^{a}$
	Sonication	$59.23\pm2.78_{a}^{c}$	$60.33\pm2.84_{a}^{b}$	$59.54\pm2.80_{a}^{b}$	$64.73\pm3.04_{a}^{a}$
	Magnetic Stirrer	$55.81\pm2.62_{b}^{c}$	$58.82\pm2.76_{b}^{b}$	$58.13\pm2.73_{b}^{b}$	$63.11\pm2.97_{b}^{a}$

age inhibition of peroxidation in linoleic acid by the extracts

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

Leaf samples of *Ficus* species probed in the present study also showed fairly good ability to inhibit peroxidation in linoleic acid under the influence of different combinations of solvent and technique used for extraction. Inhibition of the linoleic acid peroxidation by the extracts obtained from the leaf samples obeyed the general trend in most of the cases and the extract obtained by the application of 80% methanol as extraction solvent exhibited significantly ($p<0.05$) higher inhibitory effect on peroxidation of linoleic acid than the extracts obtained by the application of other solvents. Sonication assisted extraction remained significantly ($p<0.05$) more effective technique than the other techniques applied in this study for yielding extracts with potent inhibition potential. Significantly ($p<0.05$) different %age inhibitions of peroxidation of linoleic acid were shown by the extracts obtained from the leaf samples of *F. bengalensis*, *F. infectoria* and *F. religiosa* by the application of all solvents used in the present study for extraction. There was no significant ($p<0.05$) difference observed in the inhibition ability of the extracts obtained from the leaves of *F. racemosa* by the application of absolute ethanol, absolute methanol and 80% ethanol. The inhibitory effect on the peroxidation of linoleic acid was not significantly ($p<0.05$) different for the extracts obtained from the leaves of *F. retusa* by the application of absolute methanol and 80% ethanol. As for as effect of extraction techniques on the inhibition of linoleic acid peroxidation is concerned, significantly ($p<0.05$) different inhibition was observed for the leaf extracts of *F. bengalensis* and *F. racemosa* obtained by the application of different extraction techniques but no significant ($p<0.05$) difference was obtained between the extracts obtained by the application of orbital shaker and magnetic stirrer from the leaves of *F. infectoria* and *F. retusa*. No significant ($p<0.05$) difference was found between the inhibitory potential of extracts obtained by the application of magnetic stirrer and sonication from the leaves of *F. religiosa*. Overall, the extracts obtained from the leaves of *F. retusa* showed highest potential to inhibit the peroxidation in linoleic acid and the extracts obtained from the leaves of *F. religiosa* showed the lowest ability to inhibit the linoleic acid peroxidation among others.

Results calculated for the inhibition of linoleic acid peroxidation of the bark samples of selected species of *Ficus* are given in table 4.18 and these results confirm that all the bark samples studied follow the general trend as explained previously. Values of %age inhibition ranged from 59.46±2.79 to 67.57±3.18, 43.12±2.03 to 49.74±2.34, 62.07±2.92 to 69.86±3.28, 53.61±2.52 to 60.28±2.83 and 58.66±2.76 to 67.10±3.15 for the extracts obtained from barks of

Table 4.18. Effect of extraction procedure on the %age inhibition of peroxidation in linoleic acid by the extracts obtained from the barks of selected species of *Ficus*

Specie	Technique	100% Ethanol	80% Ethanol	100% Methanol	80%Methanol
F. bengalensis	Orbital Shaker	$59.46\pm2.79_{c}^{d}$	$61.45\pm2.89_{c}^{b}$	$60.72\pm2.85_{c}^{c}$	$65.93\pm3.1_{c}^{a}$
	Sonication	$61.82\pm2.91_{a}^{d}$	$62.97\pm2.96_{a}^{b}$	$62.23\pm2.92_{a}^{c}$	$67.57\pm3.18_{a}^{a}$
	Magnetic Stirrer	$60.71\pm2.85_{b}^{d}$	$61.84\pm2.91_{b}^{b}$	$61.11\pm2.87_{b}^{c}$	$66.35\pm3.12_{b}^{a}$
F. infectoria	Orbital Shaker	$43.12\pm2.03_{ab}^{c}$	$45.61\pm2.14_{ab}^{b}$	$45.07\pm2.12_{ab}^{bc}$	$48.94\pm2.3_{ab}^{a}$
	Sonication	$45.51\pm2.14_{a}^{c}$	$46.36\pm2.18_{a}^{b}$	$45.81\pm2.15_{a}^{bc}$	$49.74\pm2.34_{a}^{a}$
	Magnetic Stirrer	$44.9\pm2.11_{b}^{c}$	$45.74\pm2.15_{b}^{b}$	$45.2\pm2.12_{b}^{bc}$	$49.07\pm2.31_{b}^{a}$
F. Racemosa	Orbital Shaker	$62.07\pm2.92_{b}^{c}$	$63.22\pm2.97_{b}^{b}$	$62.39\pm2.94_{b}^{c}$	$67.14\pm3.19_{b}^{a}$
	Sonication	$63.92\pm3.00_{a}^{c}$	$65.11\pm3.06_{a}^{b}$	$64.34\pm3.02_{a}^{c}$	$69.86\pm3.28_{a}^{a}$
	Magnetic Stirrer	$62.27\pm2.92_{b}^{c}$	$63.22\pm2.97_{b}^{b}$	$62.48\pm2.94_{b}^{c}$	$67.84\pm3.19_{b}^{a}$
F. Religiosa	Orbital Shaker	$53.61\pm2.52_{c}^{d}$	$54.92\pm2.58_{c}^{b}$	$53.96\pm2.54_{c}^{c}$	$58.59\pm2.75_{c}^{a}$
	Sonication	$55.15\pm2.59_{a}^{d}$	$56.18\pm2.64_{a}^{b}$	$55.52\pm2.61_{a}^{c}$	$60.28\pm2.83_{a}^{a}$
	Magnetic Stirrer	$54.58\pm2.57_{b}^{d}$	$55.59\pm2.61_{b}^{b}$	$54.94\pm2.58_{b}^{c}$	$59.65\pm2.80_{b}^{a}$
F. retusa	Orbital Shaker	$58.66\pm2.76_{c}^{c}$	$61.27\pm2.88_{c}^{b}$	$59.05\pm2.78_{c}^{c}$	$64.11\pm3.01_{c}^{a}$
	Sonication	$61.39\pm2.89_{a}^{c}$	$62.53\pm2.94_{a}^{b}$	$61.79\pm2.90_{a}^{c}$	$67.10\pm3.15_{a}^{a}$
	Magnetic Stirrer	$60.22\pm2.83_{b}^{c}$	$61.34\pm2.88_{b}^{b}$	$60.61\pm2.85_{b}^{c}$	$65.81\pm3.09_{b}^{a}$

age inhibition of peroxidation in linoleic acid by the extracts

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column for the same specie represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

F. bengalensis, F. Infectoria, F. racemosa, F. religiosa and *F. retusa* respectively. All the upper and lower values of each range were significantly ($p<0.05$) different from each other. Effect of solvent on the %age inhibition of linoleic acid of the extracts obtained from *F. bengalensis, F. Infectoria* and *F. religiosa* was significantly ($p<0.05$) different and the statistical ranking of the solvents on the basis of their effect on the %age inhibition was 80%methanol> 80% ethanol> 100% methanol> 100% ethanol. The ranking of solvents on the basis of their effect on the %age inhibition of the extracts obtained from the barks of *F. racemosa* and *F. retusa* was 80%methanol> 80% ethanol> 100% methanol ≈ 100% ethanol. The techniques significantly ($p<0.05$) effect the %age inhibition of the extracts acquired from the barks of *F. bengalensis, F. Infectoria, F. religiosa* and *F. retusa* and their statistical ranking was sonication > magnetic stirring > orbital shaking. The ranking of techniques for the bark extracts of *F. racemosa* was sonication > magnetic stirring ≈ orbital shaking. Regardless of the choice of solvent and technique employed, when compared the %age inhibition values among bark samples from different species selected, it was noted that the extracts from the bark samples of *F. racemosa* had the highest ability to inhibit the linoleic acid peroxidation followed by bark extracts of *F. bengalensis,* bark extracts from *F. retusa*, bark samples of *F. religiosa* and *F. Infectoria*.

Tert-butyl-1- hydroxytoluene (BHT) was used as positive control in the present study and its %age inhibition value was equal to 73.06±0.14 which was lower than the %age inhibition value of the extracts obtained from the fruits of *F. retusa* by the application of 80% methanol with all the three techniques. Reducing power of the extracts obtained from the fruits of *F. bengalensis* and *F. racemosa* by employing 80% methanol was comparable to the %age inhibition value of BHT while the %age inhibition value of other samples investigated in the present study was lower than that of BHT.

Sultana *et al.*, (2009) reported %age inhibition of linoleic acid for the fruit extracts of *F. religiosa* with orbital shaking as extraction technique and with same four solvents as we used in the present study and reported values of %age inhibition of linoleic acid peroxidation of the fruit extract were 67.4 ±2.1% (with 80% methanol), 60.8 ± 2.4 (with 80% ethanol), 59.2 ± 1.7(with 100% methanol), 54.9 ± 2.1 (with 100% ethanol). These reported values are in close agreement with our determined values 66.8±3.14 (with 80% methanol), 63.21±2.97 (with 80% ethanol), 61.53±2.89 (with 100% methanol) and 59.27±2.79 (with 100% ethanol) for the fruit of same plant with same solvent and same technique. The trend mentioned by Sultana *et al.*, (2009) for the efficiency of solvents on the basis of their ability to inhibit the peroxidation of linoleic acid was same as we investigated in our study. Shi *et al.*, (2011) investigated the ability of the leaves of seven *Ficus* species to inhibit the peroxidation of linoleic acid and reported maximum %age inhibition value 83.8% and minimum value 41.4% and this range was much higher than the %age inhibition range (27±1.27% to 59.54±2.8%) determined for the leaf samples investigated in the present study by using absolute methanol as solvent. Rathee *et al.*, (2010) reported %age inhibition of lipid peroxidation for the methanolic fruit extract of *F. religiosa* at different concentrations and it was 61.74% at concentration of 1mg/mL which was in close agreement with our calculated value of 61.53±2.89% for the methanolic extract of *F. religiosa* at the same concentration level.

4.7. Correlation analysis

Correlation analysis explains the dependence of different independent variables on each other. Many reports have been published showing the correlation analysis between TPC, TFC and other antioxidant activities (Manzoor *et al.*, 2012). Different secondary metabolites, occurring in botanical sources especially phenolic acids and flavonoids, are thought to be responsible for different bioactivities of plants. The reducing potential and radical scavenging activities of plant materials are also ascribed to the occurrence of phenolic compounds in plants. Hence the correlation analysis among the concentrations of TPC, TFC and different bioactivities of plants can be understood.

The results for correlation analysis among TPC, TFC and different antioxidant activities including DPPH radical scavenging activity ($1/IC_{50}$ values were used for comparison purpose), reducing power and %age inhibition of linoleic acid peroxidation was conducted and represented in table 4.19 for fruit, in table 4.20 for leaf and in table 4.21 for bark samples of selected species of *Ficus*.

In case of fruit samples of all the *Ficus* species, correlation analysis revealed that TFC and different bioactivities including DPPH radical scavenging activity, reducing power and %age inhibition of linoleic acid peroxidation were significantly correlated with the amount of TPC. The correlation coefficients (*r*) between TPC and TFC of all the fruit samples except for the fruits of *F. infectoria* were highly significant (values of *r* were 0.833***, 0.953***, 0.971*** and 0.972*** for the fruits of *F. bengalensis*, *F. racemosa*, *F. religiosa* and *F. retusa*, respectively) but for the fruits of *F.*

infectoria value of *r* was 0.312^{ns} (non-significant) and the same trend was found for the values of correlation coefficient (0.949***, 0.912***, 0.977*** and 0.972*** for the fruits of *F. bengalensis, F. racemosa, F. religiosa* and *F. retusa*, respectively) obtained after the correlation analysis between TPC and DPPH. In case of fruits of *F. infectoria* correlation between TPC and DPPH (*r* =0.501^{ns}) was non-significant. Correlation between TPC and reducing power (*r*=0.95***, 0.899***, 0.996***, 0.998*** and 0.99*** for the fruits of *F. bengalensis, F.infectoria, F. racemosa, F. religiosa* and *F. retusa*, respectively) was found to be highly significant for all the fruit samples of *Ficus* species investigated presently. Similarly %age inhibition of linoleic acid peroxidation activity of the fruit samples also exhibited highly significant dependence on the quantity of TPC except for the fruits of *F. infectoria* where significant level was low (*r*=0.588*). The results of correlation analysis also revealed that antioxidant activities of fruit samples are also significantly dependent on the quantity of TFC. Correlation coefficient (*r*) values for correlation between TFC and DPPH radical scavenging activity (1/IC_{50}) were found equal to 0.798**, 0.869***, 0.931***, 0.972*** and 0.988*** for the fruits of *F. bengalensis, F.infectoria, F. racemosa, F. religiosa* and *F. retusa,* respectively and these values of correlation coefficient revealed that antioxidant activities were highly dependent on the amounts of TFC determined in the present study. Correlation coefficient (*r*) values for correlation between TFC and reducing power were found equal to 0.964***, 0.695*, 0.974***, 0.984*** and 0.995*** for the fruits of *F. bengalensis, F.infectoria, F. racemosa, F. religiosa* and *F. retusa*, respectively and these values clearly indicate that reducing power of the fruit extracts is significantly correlated with the amount of TFC. Similar trend was shown for correlation between TFC and % age inhibition of linoleic acid peroxidation (*r*= 0.977***, 0.871***, 0.953***, 0.883*** and 0.909*** for the fruits of *F. bengalensis, F.infectoria, F. racemosa, F. religiosa* and *F. retusa*, respectively) and these values of coefficient correlation clearly indicate that both these parameters are significantly correlated with each other. Correlation between 1/IC_{50} and reducing power (*r*=0.905***, 0.779**, 0.924***, 0.981*** and 0.988*** for the fruits of *F. bengalensis, F.infectoria, F. racemosa, F. religiosa* and *F. retusa*, respectively) was also found to be significant. Similarly correlation

Table 4.19. Correlation between different antioxidant assays of fruit samples of *Ficus* species represented by correlation coefficient (r)

F. bengalensis		TPC	TFC	1/IC_{50}	Reducing power	%age Inhibition

	TPC	1				
	TFC	0.833***	1			
	$1/IC_{50}$	0.949***	0.798**	1		
	Reducing power	0.95***	0.964***	0.905***	1	
	%age Inhibition	0.977***	0.977***	0.95***	0.95***	1
F. infectoria	TPC	1				
	TFC	0.312^{ns}	1			
	IC50	0.501^{ns}	0.869***	1		
	Reducing power	0.899***	0.695*	0.779**	1	
	%age Inhibition	0.588*	0.871***	0.97***	0.959***	
F. racemosa	TPC	1				
	TFC	0.953***	1			
	$1/IC_{50}$	0.912***	0.931***	1		
	Reducing power	0.996***	0.974***	0.924***	1	
	%age Inhibition	0.911***	0.953***	0.947***	0.93***	1
F.religiosa	TPC	1				
	TFC	0.971***	1			
	$1/IC_{50}$	0.977***	0.972***	1		
	Reducing power	0.998***	0.984***	0.981***	1	
	%age Inhibition	0.944***	0.883***	0.952***	0.935***	1
F.retusa	TPC	1				
	TFC	0.973***	1			
	$1/IC_{50}$	0.972***	0.988***	1		
	Reducing power	0.99***	0.995***	0.988***	1	

	%age Inhibition	0.942***	0.909***	0.95***	0.93***	1

Table 4.20. Correlation between different antioxidant assays of leaf samples of *Ficus* species represented by correlation coefficient (r)

		TPC	TFC	IC50	Reducing power	%age Inhibition
F. bengalensis	TPC	1				
	TFC	0.975***	1			
	IC50	0.998***	0.985***	1		
	Reducing power	0.999***	0.98***	0.999***	1	
	%age Inhibition	0.812**	0.879***	0.831***	0.822**	
F. infectoria	TPC	1				
	TFC	0.765**	1			
	IC50	0.977***	0.87***	1		
	Reducing power	0.99***	0.843***	0.994***	1	
	%age Inhibition	0.947***	0.827***	0.949***	0.962***	1
F. racemosa	TPC	1				
	TFC	0.939***	1			
	IC50	0.983***	0.985***	1		
	Reducing power	0.991***	0.976***	0.998***	1	
	%age Inhibition	0.669*	0.647*	0.672*	0.669*	1
F.religiosa	TPC	1				
	TFC	0.819**	1			
	IC50	0.891***	0.975***	1		
	Reducing power	0.945***	0.96***	0.891***	1	

	%age Inhibition	0.899***	0.764**	0.975***	0.861	1
F.retusa	TPC	1				
	TFC	0.975***	1			
	IC50	0.96***	0.984***	1		
	Reducing power	0.996***	0.989***	0.976***	1	
	%age Inhibition	0.815**	0.875***	0.816**	0.837***	1

Table 4.21. Correlation between different antioxidant assays of bark samples of *Ficus* species represented by correlation coefficient (r)

		TPC	TFC	IC50	Reducing power	%age Inhibition
F. bengalensis	TPC	1				
	TFC	0.963***	1			
	IC50	0.998***	0.978***	1		
	Reducing power	0.997***	0.98***	0.999***	1	
	%age Inhibition	0.902***	0.936***	0.913***	0.919***	1
F. infectoria	TPC	1				
	TFC	0.98***	1			
	IC50	0.994***	0.99***	1		
	Reducing power	0.998***	0.986***	0.995***	1	
	%age Inhibition	0.907***	0.868***	0.872***	0.91***	1
F. racemosa	TPC	1				
	TFC	0.997***	1			
	IC50	0.998***	0.999***	1		
	Reducing power	0.999***	0.998***	0.999***	1	
	%age Inhibition	0.819**	0.802**	0.795**	0.816***	1
F.religiosa	TPC	1				

	TFC	0.996***	1			
	IC50	0.998***	0.998***	1		
	Reducing power	0.999***	0.997***	0.998***	1	
	%age Inhibition	0.9***	0.878***	0.876***	0.999***	1
F.retusa	TPC	1				
	TFC	0.975***	1			
	IC50	0.999***	0.976***	1		
	Reducing power	0.999***	0.981***	0.999***	1	
	%age Inhibition	0.883***	0.928***	0.887***	0.893***	1

between $1/IC_{50}$ and %age inhibition of linoleic acid peroxidation was also found to be quite significant.

In case of leaf and bark samples of the selected species of *Ficus*, correlation between all the parameters was found highly significant except for the correlation between TPC and %age inhibition of linoleic acid peroxidation (r=0.669*), TFC and %age inhibition of linoleic acid peroxidation (r=0.647*), $1/IC_{50}$ and %age inhibition of linoleic acid peroxidation (r=0.672*) and reducing power and %age inhibition of linoleic acid peroxidation (r=0.669*) for the leaf samples of *F. racemosa*, although correlation between these parameters was significant but level of significance was lower. Ao *et al.*, (2008) also evaluated correlation between TPC and different antioxidant assays ($ABTS^+$ assay, DPPH assay, PMS-NDH and b-carotene bleaching effect) and reported correlation coefficient values 0.919 for TPC and $ABTS^+$, 0.836 for correlation between TPC and DPPH, 0.813 for correlation between TPC and PMS−NADH and correlation between

TPC and b-carotene bleaching method was 0.534.

These values show significant correlation between TPC and antioxidant capacities of *F. microcarpa* . Similar results for the correlation between TPC and different antioxidant assays were reported by other authors (Gorinstein *et al.*, 2003; Maleeha *et al.*, 2012; Mai-suthisakul, *et al.*, 2007). Although these reports and results for correlation analysis represented in this study agreed that antioxidant activities of plants are correlated with the concentration of phenolic compounds but the compounds other than phenolics can contribute to the antioxidant activities of extracts obtained from botanical sources (Ao *et al.*, 2008) and this may be the cause of contradictory results for correlation analysis among different antioxidant assays performed on the fruit samples of *F. infectoria*.

4.8. Identification and quantification of phenolic acids by RP-HPLC

All the samples of fruit, leaves and bark from the selected species of *Ficus* exhibited fairly good amounts of total pheolics and total flavonoids but the extracts obtained by using 80% methanol and sonication as extraction technique showed the highest level of total phenolics and total flavonoids among others. Hence the extracts of the samples obtained by using the 80% methanol as extraction solvent and sonication as extraction technique were further analyzed to identify and quantify their individual phenolic acids and flavonoids. For this purpose these extracts were hydrolyzed to convert bound form phenolic into their free form (aglycones). Then these samples after filtration were subjected to HPLC analysis for separation of targeted phenolic acids and flavonoid compounds followed by their identification and quantification. Caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, gallic acid, gentisic acid, , protocatechuic acid, sinapic acid, syringic acid and vanillic acid were used as standard compounds for the identification, and calibration purposes as well as to quantify their amounts.

Phenolic acids identified and quantified (mg/100g DW) from the fruits of selected species of *Ficus* are summarized in table 4.22. Gallic acid was not detected in any of the fruit sample investigated in the present study while gentisic acid, chlorogenic acid, caffeic acid and sinapic acid were identified in all the fruit samples of *Ficus* species investigated in the present study.

Significantly ($P<0.05$) higher amount of gentisic acid was found in fruits of *F. retusa*

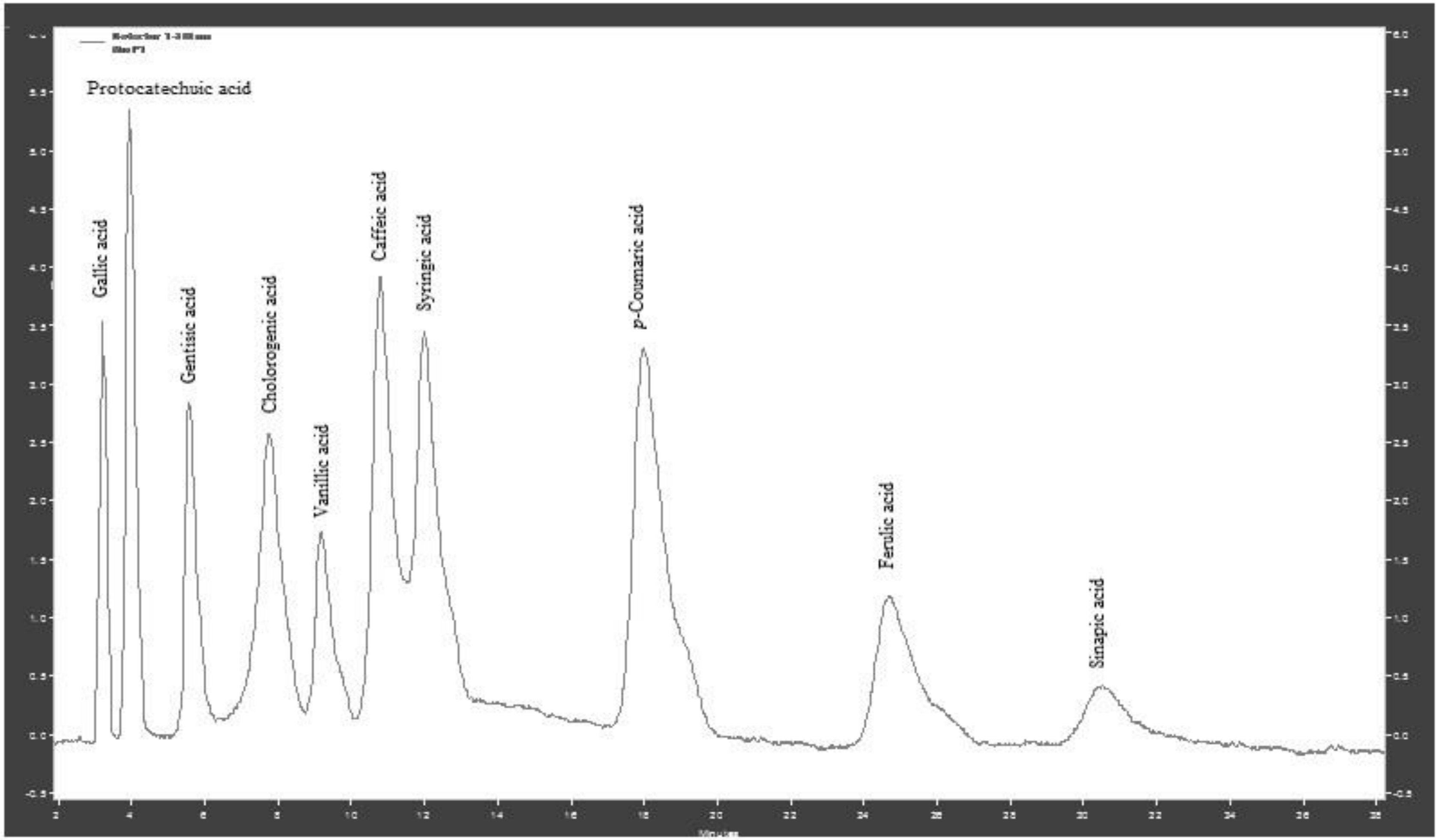


Figure 4.1. HPLC chromatogram, showing separation of pure phenolic acids

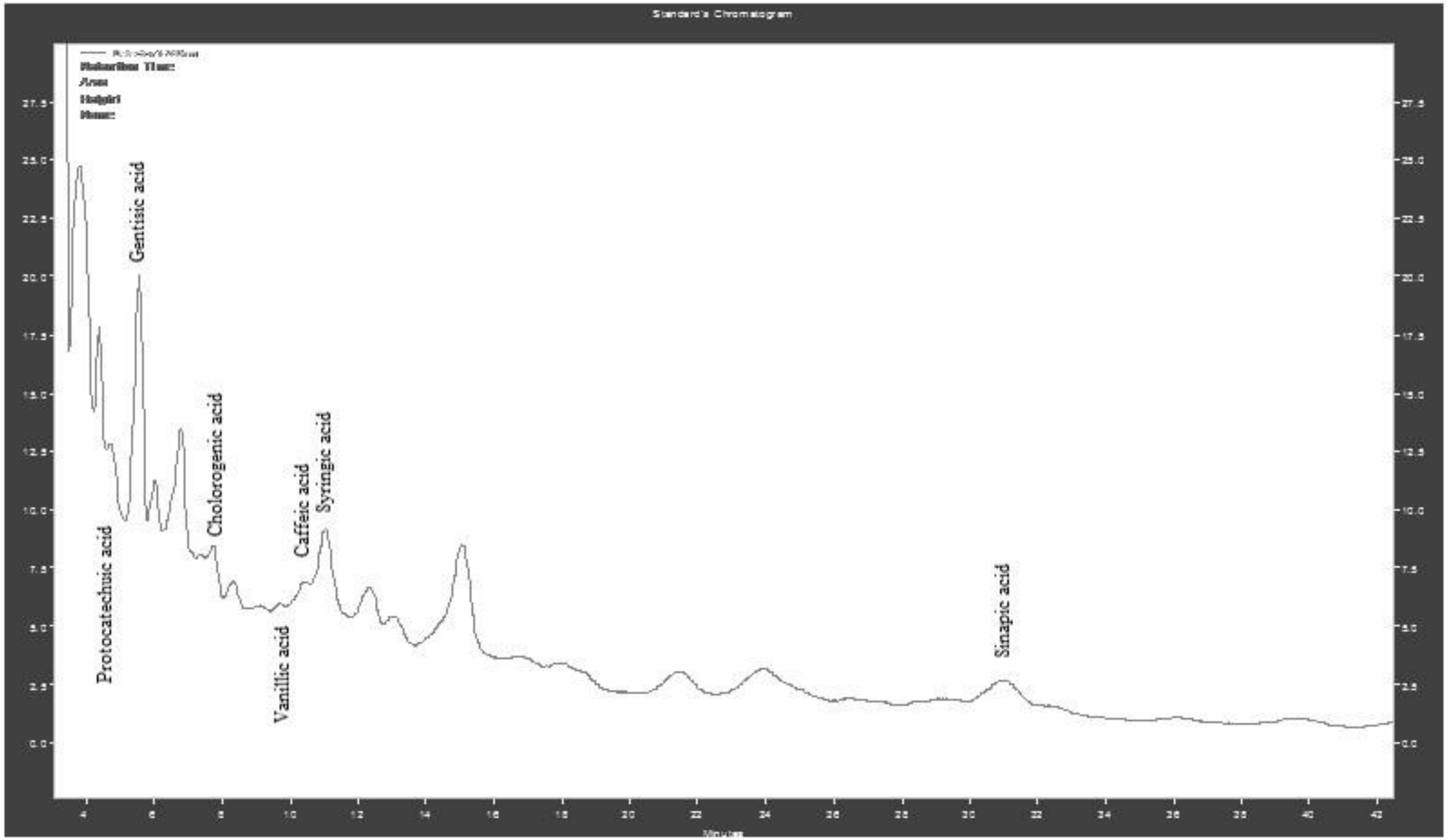

Standard's Chromatogram
Gentisic acid
Protocatechuic acid
Cholorogenic acid
Vanillic acid
Caffeic acid
Syringic acid
Sinapic acid
Minutes

Figure 4.2. A typical chromatogram, showing separation of phenolic acids from the fruits of *F. bengalensis*

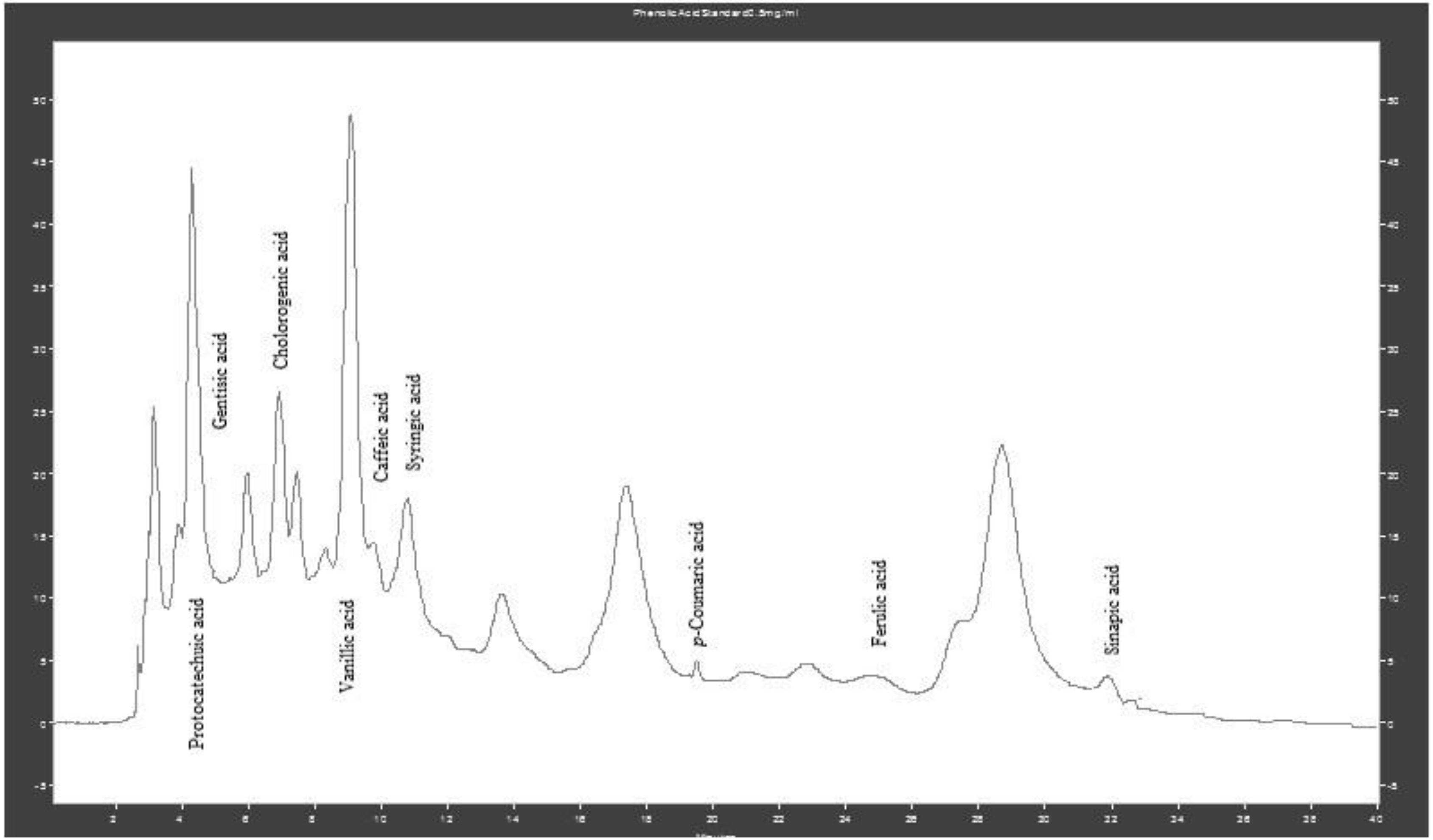


Figure 4.3. A typical chromatogram, showing separation of phenolic acids from the leaves of *F. religiosa*

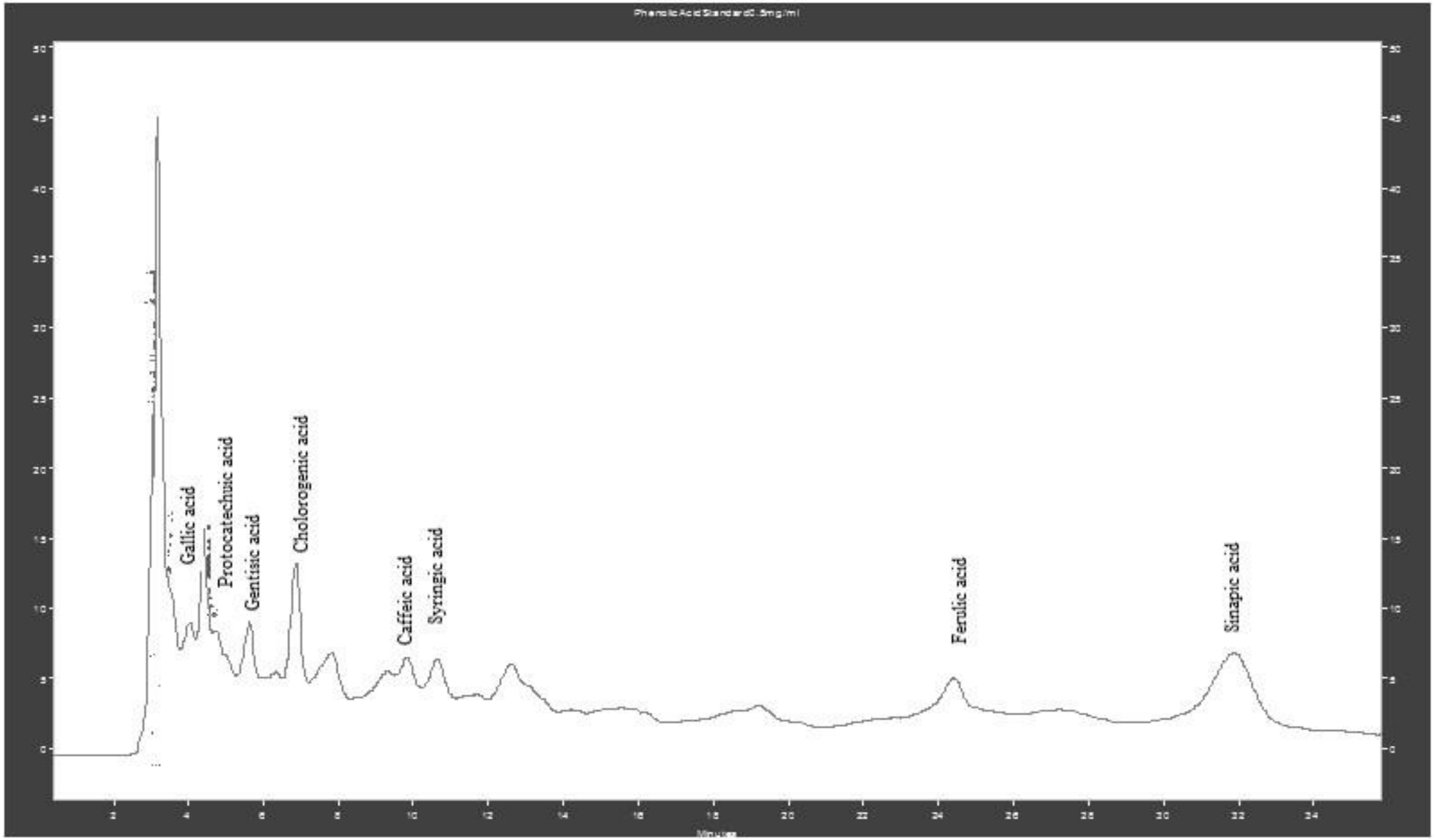


Figure 4.4. A typical chromatogram, showing separation of phenolic acids from the bark of *F. infectoria*

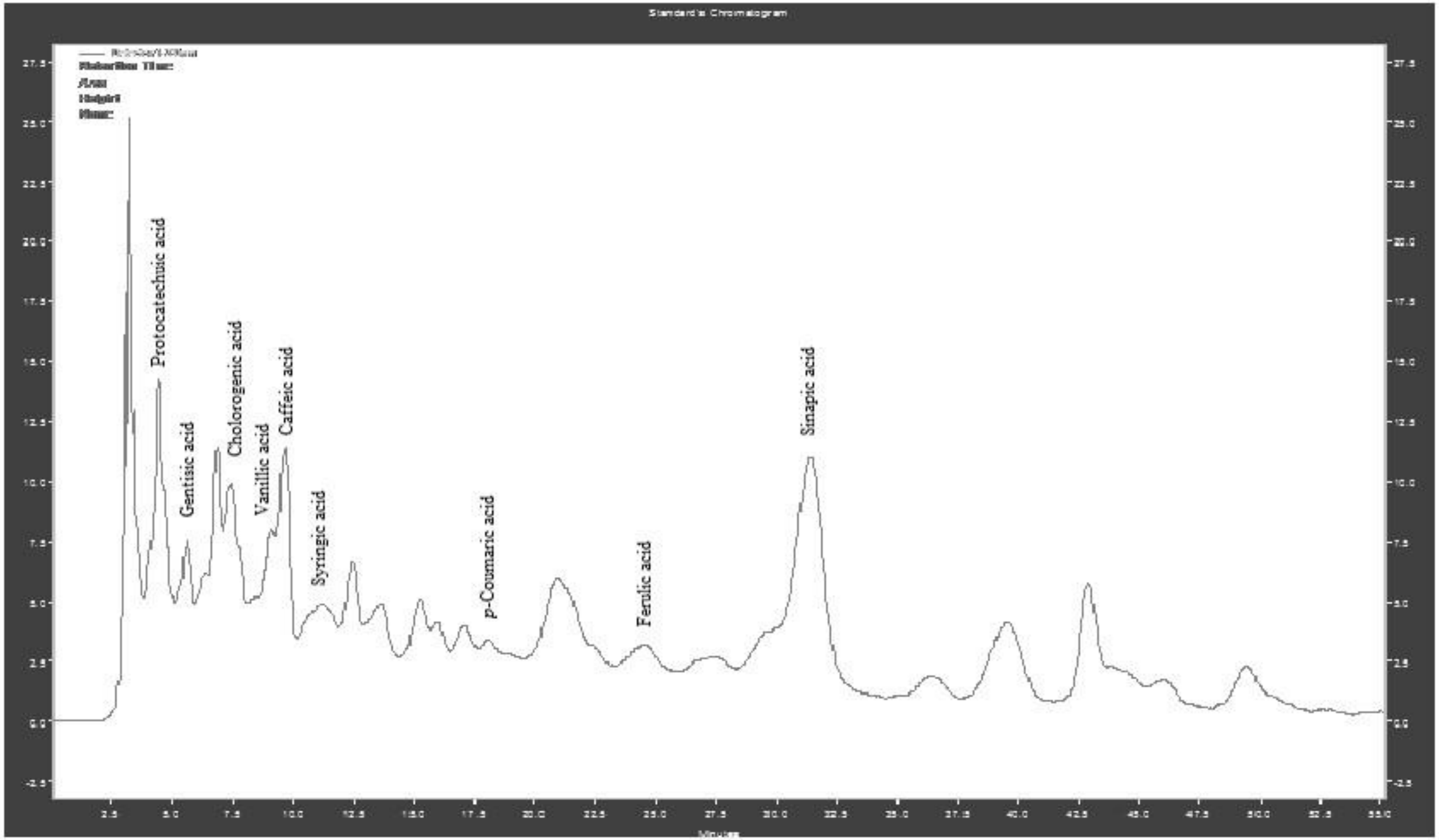


Figure 4.5. A typical chromatogram, showing separation of phenolic acids from the bark of *F. retusa*

Table 4.22. Phenolic acids (mg/100g of dried sample) quantified by HPLC from the fruits of selected species of *Ficus*

Name	Gallic acid	Protocatechuic acid	Gentisic acid	Chlorogenic acid	Vanillic acid	Caffeic acid	Syringic acid	p-Coumaric acid	Ferulic acid	Sinapic acid
F. bengalensis	ND	$2.40\pm0.12_{c}$	$6.12\pm0.20_{c}$	$0.75\pm0.03_{d}$	$1.26\pm0.06_{b}$	$3.68\pm0.16_{d}$	$1.48\pm0.06_{c}$	ND	ND	$0.68\pm0.03_{e}$
F. infectoria	ND	$2.77\pm0.12_{c}$	$6.70\pm 0.50_{c}$	$1.49\pm0.07_{c}$	ND	$9.56\pm0.38_{b}$	ND	$4.57\pm0.19_{c}$	ND	$63.5\pm2.43_{a}$
F. racemosa	ND	$9.96\pm0.40_{a}$	$34.30\pm0.99_{b}$	$8.83\pm0.34_{b}$	ND	$2.69\pm0.26_{e}$	$2.86\pm0.02_{b}$	$14.32\pm0.67_{b}$	$0.37\pm0.01_{c}$	$8.14\pm0.03_{d}$
F. religosa	ND	$6.64\pm0.25_{b}$	$2.10\pm0\ .24_{d}$	$1.46\pm0.06_{c}$	$0.84\pm0.04_{c}$	$7.69\pm0.29_{c}$	$4.90\pm0.20_{a}$	$4.38\pm0.22_{c}$	$6.92\pm0.27_{a}$	$14.86\pm0.58_{c}$
F. retusa	ND	ND	$40.0\pm1.24_{a}$	$18.11\pm0.73_{a}$	$8.37\pm0.85_{a}$	$11.12\pm0.89_{a}$	ND	$35.06\pm1.30_{a}$	$5.64\pm0.24_{b}$	$23.93\pm1.09_{b}$

LOD	0.02	0.01	0.02	0.03	0.04	0.02	0.02	0.02	0.04	0.05

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column represent different significance levels ($p \leq 0.05$) of phenolic acid among different species investigated by LSD (least significant difference) test, ***LOD=Limit of detection in mg/Liter.

Table 4.23. Phenolic acids (mg/100g of dried sample) quantified by HPLC from the leaves of selected species of *Ficus*

Name	Gallic acid	Protocatechuic acid	Gentisic acid	Chlorogenic acid	Vanillic acid	Caffeic acid	Syringic acid	p-Coumaric acid	Ferulic acid	Sinapic acid
F. bengalensis	$5.21\pm0.23_{a}^{d}$	$7.63\pm0.4_{d}^{c}$	$16.70\pm0.59_{a}^{b}$	$21.93\pm1.96_{b}^{a}$	$4.93\pm0.22_{e}^{d}$	$2.29\pm0.09_{a}^{e}$	ND	$2.51\pm0.09_{c}^{e}$	$2.72\pm0.12_{a}^{e}$	$4.57\pm0.18_{b}^{d}$
F. infectoria	$4.61\pm0.19_{b}$	$9.40\pm0.39_{c}$	$5.51\pm0.24_{c}$	$6.68\pm.35_{d}$	$23.68\pm0.78_{b}$	$1.38\pm0.07_{c}$	$4.74\pm0.09_{a}$	$8.84\pm0.31_{a}$	$1.36\pm0.52_{b}$	$3.54\pm0.16_{c}$
F. racemosa	ND	$28.47\pm1.5_{a}$	$1.98\pm0.08_{e}$	$25.091\pm1.04_{a}$	$26.87\pm1.12_{a}$	$1.27\pm0.06_{c}$	$1.74\pm0.05_{c}$	$5.22\pm0.22_{b}$	2.62±0.41a	0.61±0.03d
F. religosa	ND	$11.16\pm0.49_{b}$	$3.14\pm0.12_{d}$	$5.13\pm0.16_{e}$	$9.86\pm0.43_{d}$	$1.05\pm0.05_{d}$	$3.52\pm0.12_{b}$	$0.36\pm0.01_{d}$	$1.26\pm0.04_{b}$	$0.91\pm0.04_{d}$

F. retusa	4.52±0.17$_b$	5.16±0.21$_e$	7.23±0.24$_b$	8.67±.53$_c$	11.62±0.4$_c$	1.54±0.07$_b$	1.17±0.04$_d$	0.26±0.01$_d$	1.06±0.05$_c$	8.80±0.33$_a$
LOD	0.02	0.01	0.02	0.03	0.04	0.02	0.02	0.02	0.04	0.05

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column represent different significance levels ($p \leq 0.05$) of phenolic acid among different species investigated by LSD (least significant difference) test, ***LOD= Limit of detection in mg/Liter

Table 4.24: Phenolic acids (mg/100g of dried sample) quantified by HPLC from the barks of selected species of *Ficus*

Name	Gallic acid	Protocatechuic acid	Gentisic acid	Chlorogenic acid	Vanillic acid	Caffeic acid	Syringic acid	p-Coumaric acid	Ferulic acid	Sinapic acid
F. bengalensis	0.26±0.01$_c$	0.67±0.03$_d$	2.14±0.08$_c$	0.62±0.03$_c$	0.97±0.05$_b$	0.33±0.01$_d$	0.1±0.01$_c$	0.03±0.01$_d$	ND	6.69±0.29$_b$
F. infectoria	0.73±0.03$_b$	0.16±0.01$_d$	2.70±0.11$_c$	1.75±0.08$_c$	ND	0.45±0.02$_d$	0.6±0.02$_b$	ND	0.24±0.02$_b$	2.22±0.08$_d$
F. racemosa	2.24±0.09$_a$	43.04±1.70$_b$	1.29±0.05$_d$	29.5±1.20$_b$	ND	3.78±0.16$_b$	ND	0.94±0..04$_b$	ND	3.06±0.11$_c$

F. religiosa	$2.26\pm0.10_{a}$	$56.03\pm2.22_{a}$	$14.9\pm0.51_{a}$	$40.87\pm1.60_{a}$	ND	$5.06\pm0.21_{a}$	ND	$1.39\pm0.06_{a}$	ND	$2.64\pm0.09_{cd}$
F. retusa	ND	$3.94\pm0.18_{c}$	$3.72\pm0.12_{b}$	$2.63\pm0.12_{c}$	$2.42\pm0.09_{a}$	$1.04\pm0.04_{c}$	$1.38\pm0.06_{a}$	$0.19\pm0.02_{c}$	$1.27\pm0.07_{a}$	$11.36\pm0.46_{a}$
LOD	0.02	0.01	0.02	0.03	0.04	0.02	0.02	0.02	0.04	0.05

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column represent different significance levels (p ≤ 0.05) of phenolic acid among different species investigated by LSD (least significant difference) test, ***LOD= Limit of detection in mg/Liter

(40.0±1.24) followed by *F. racemosa* (34.30±0.99), *F. infectoria* (6.70± 0.50) ≈ *F. bengalensis* (6.12±0.20) and *F. religosa* (2.10±0 .24). Presence of chlorogenic acid in the fruit samples was ranged from 0.75±0.03 (*F. bengalensis*) to 18.11±0.73 (*F. retusa*) mg/100 g of dry matter and the amounts of chlorogenic acid found in all the fruit samples studied were significantly ($P<0.05$) different from one another. Among all the fruit samples, fruits of *F. retusa* contains significantly ($P<0.05$) higher amount (18.11±0.73) of chlorogenic acid followed by *F. racemosa* (8.83±0.34), *F. infectoria* (1.49±0.068) ≈ *F. religosa* (1.46±0.06) and *F. bengalensis* (0.75±0.03). The ranking of fruit samples studied on the basis of caffeic acid contents after statistical analysis was *F. retusa* (11.12±0.89)> *F. infectoria* (9.56±0.38)> *F. religosa* (7.69±0.29)> *F. bengalensis* (3.68±0.16)> *F. racemosa* (2.69±0.26). Significantly ($P<0.05$) different amounts of sinapic acid were detected in the fruit samples studied and significantly ($P<0.05$) higher amount was detected in the fruits of *F. infectoria* (63.5±2.43) which was followed by *F. retusa* (23.93±1.09), *F. religosa* (14.86±0.58), *F. racemosa* (8.14±0.03) and *F. bengalensis* (0.68±0.03). Protocatechuic acid was not detected in the fruit sample of *F. retusa* but was present in other fruit samples analyzed. The amounts of Protocatechuic acid detected in the fruits of selected species of *Ficus* ranged from 2.40±0.12 to 9.96±0.40 mg/100g of dried sample. Significantly ($P<0.05$) higher amount of Protocatechuic acid was found in *F. racemosa* (9.96±0.40), followed by *F. religosa* (6.64±0.25) and *F. infectoria* ≈ *F. bengalensis* (2.77±0.12-2.40±0.12). Significantly ($P<0.05$) different amounts of vanillic acid were present in the fruits of *F. retusa* (8.37±0.85), *F. bengalensis* (1.26±0.06) and *F. religiosa* (0.84±0.04) while it was not detected in the fruit samples of *F. infectoria* and *F. racemosa*. Syringic acid was not detected in the fruit samples of *F. infectoria* and *F. retusa* but significantly different amounts were identified in the fruits samples of *F. religiosa* (4.90±0.20), *F. racemosa* (2.86±0.02) and *F. bengalensis* (1.48±0.06). All the fruit samples except the fruits of *F. bengalensis* contain p-Coumaric acid ranging from 35.06±1.30 (*F. retusa*) to 4.38±0.22 (*F. religiosa*). Quantities of p-Coumaric acid detected in the fruits of *F. religiosa* and *F. infectoria* were not significantly different from one another. Significantly ($P<0.05$) different amounts of ferulic acid were recognized in the fruit samples of *F. religiosa* (6.92±0.27), *F. retusa* (5.64±0.24) and *F. racemosa* (0.37±0.01) but not found in the fruit samples of *F. bengalensis* and *F. infectoria*.

Results obtained after the HPLC study of leaves of selected species of *Ficus* are presented in table 4.23. Caffeic acid, chlorogenic aicd, ferulic acid, gentisic acid, Sinapic acid, p-coumaric acid, protocatechuic acid and vanillic acid were identified in the leaf samples of all the *Ficus* species investigated in the present study. Gallic acid was not detected in the leaf samples of *F. racemosa* and *F. religiosa* while syringic acid was not identified in the leaves of *F. bengalensis*. The highest amount of gallic acid (5.2±0.23) among the leaf samples was detected in *F. bengalensis* which was significantly (*P*<0.05) different from the quantities of gallic acid found in *F. infectoria* (4.61±0.19) and *F. retusa* (4.52±0.17). Significantly (*P*<0.05) different quantities of protocatechuic acid were detected in the leaf samples of different species of *Ficus* tested in the present study ranging from 5.16±0.21 (*F. retusa*) to 28.47±1.5 (*F. racemosa*). Among the leaf samples, significantly (*P*<0.05) higher quantity of gentisic acid (16.7±0.59) was determined in the leaves of *F. bengalensis* followed by *F. retusa* (7.23±0.24), *F. infectoria* (5.51±0.24), *F. religiosa* (3.14±0.12) and *F. racemosa* (1.98±0.08). The range of cholorogenic acid in the leaf samples was from 5.13±0.16 (*F. retusa*) to 25.09±1.04 (*F. racemosa*) and the amounts of cholorogenic acid detected in leaf samples were significantly (*P*<0.05) different from one another. The highest (26.87±1.12) and the lowest (4.93±2.17) amounts of vanillic acid among the leaf samples were found in *F. racemosa* and *F. bengalensis*, respectively. The quantities of vanillic acid detected in the leaf samples were significantly (*P*<0.05) different from each other. Minimum and maximum amount of caffeic acid in the leaf samples was detected in the leaves of *F. religiosa* (1.05±0.05) and *F. bengalensis* (2.29±0.09) respectively. The amounts of caffeic acid found in the leaves of *F.infectoria* (1.38±0.07) *and F. racemosa* (1.27±0.06) were not significantly (*P*<0.05) different although amounts of caffeic acid were significantly (*P*<0.05) different in other leaf samples. Syringic acid was detected in significantly (*P*<0.05) different quantities in the leaves of *F. infectoria* (4.74±0.09), *F. religiosa* (3.52±0.12), *F. racemosa* (1.74±0.05) and *F. retusa* (1.17±0.04) but was not detected in the leaves of *F. Bengalensis*. The range of p- coumaric acid in the leaf samples was from 0.26±0.01 (*F. retusa)* to 8.84±0.31 (*F. infectoria*). The amounts of p- coumaric acid in the leaves of *F. retusa* and *F. religiosa* were not significantly (*P*<0.05) different but amounts of p- coumaric acid were significantly (*P*<0.05) different in other leaf samples. Range of ferulic acid detected in the leaf samples was from 1.06±0.05 (*F. retusa*) to 2.72±0.12 (*F. bengalensis*). There was no significant (*P*<0.05) difference observed between the quantities of ferulic acid found in the leaves of *F. bengalensis* and *F. racemosa* and the quantities of ferulic acid detected in the leaves of *F. infectoria* and *F. religiosa* were also have no significant (*P*<0.05) difference. The highest and the lowest quantities of sinapic acid among the leaf samples were detected in the leaves of *F. retusa* (8.8±0.33) and *F. racemosa* (0.61±0.028) respectively. There was significant (*P*<0.05) difference among the amounts of sinapic acid detected in the leaf samples except those of *F. racemosa* and *F. religiosa*.

Phenolic acids and their quantities determined in the bark samples of *Ficus* species investigated in the present study are presented in tables 4.24. Protocatechuic acid, gentisic acid, chlorogenic acid, caffeic acid and sinapic acid were present in all the bark samples of selected species of *Ficus*. Gallic acid was not detected in the samples of *F. retusa*. vanillic acid was not found in the barks of *F.infectoria*, *F.racemosa* and *F. religiosa*, p-coumaric acid was not found in the bark of *F.infectoria* and ferulic acid was not detected in the barks of *F. bengalensis*, *F.racemosa* and *F. religiosa*. In the present study, Protocatechuic acid was in the highest amount (56.03±22) among all the other phenolic acids identified in the bark samples while p-Coumaric acid was detected in lowest amount (0.029±0.01) among all the phenolic acids identified in the samples scrutinised in the present study. In most of the cases, there was a significant (*P*<0.05) variance (*P*<0.05) in the amounts of a phenolic acids evaluated in different bark samples of *Ficus*. Among the bark samples, the highest (2.26±0.1) and the lowest (0.26±0.11) amounts of gallic acid were quantified in the barks of *F. religiosa* and *F. bengalensis*, respectively. No significant (*P*<0.05) difference was detected between the amounts of gallic acid detected in the bark of *F. religiosa* and *F.racemosa* but the quantities of gallic acid detected in other samples were significantly (*P*<0.05) different from each other. protocatechuic acid was detected in all the bark samples studied ranging from 0.16±0.074 (*F. Infectoria*) to 56.03±22 (*F. religiosa*) and protocatechuic acid was detected in significantly (*P*<0.05) different quantities in the bark samples of *F. religiosa*, *F.racemosa* and *F. retusa* but there was no significant (*P*<0.05) difference between the quantities of protocatechuic acid found in barks of *F. bengalensis* and *F. Infectoria*. In the bark samples, gentisic acid ranged from1.29±0.52 (*F. racemosa*) to 14.9±0.51 (*F. religiosa*) and the amounts of gentisic acid in the bark samples of *F. bengalensis* and *F. Infectoria* have no significant (*P*<0.05) difference but the amounts of gentisic acid detected in other bark samples were significantly (*P*<0.05) different from each other. Among the bark samples, significantly (*P*<0.05) higher amount of chlorogenic acid was detected in the bark of *F. religiosa* (40.87±1.6) followed by *F.racemosa* (29.5±1.20) and *F. retusa* (2.63±0.12) ≈ *F. Infectoria*

(1.75±0.08) ≈ *F. Bengalensis* (0.62±0.03). vanillic acid was detected only in the bark samples of *F. retusa* (2.42±0.09) and *F. Bengalensis* (0.97±0.48) and both these amounts were significantly (*P*<0.05) different. Caffeic acid was detected in the range of 0.33±0.014 (*F. bengalensis*) to 5.06±0.21 (*F. religiosa*) in the bark samples. The ranking of bark samples on the basis of caffiec acid contents from higher to lower is *F. religiosa* (5.06±0.21) > *F.racemosa* (3.78±0.16) > *F. retusa* (1.04±0.04) > *F. Infectoria* (0.45±0.02) ≈ *F. bengalensis* (0.33±0.014). Among the barks of tested species, syringic acid was detected only in *F. retusa* (1.38±0.58), *F. Infectoria* (0.6±0.02) and *F. bengalensis* (0.1±0.04) and all these amounts were significantly (*P*<0.05) different from one another. The range of p-coumaric acid in the bark sample was from 0.03±0.01 (*F. Bengalensis*) to 1.39±0.67 (*F. religiosa*) and it was found in significantly (*P*<0.05) different levels in the barks of *F. religiosa*, *F.racemosa*, *F. retusa* and *F. Bengalensis* but not detected in the bark of *F. Infectoria*. Among the bark samples, ferulic acid was detected only in the barks of *F. retusa* (1.27±0.57) and *F. infectoria* (0.24±0.09) and both these amounts were significantly (*P*<0.05) different from each other. Significantly (*P*<0.05) different quantities of sinapic acid were detected in all the bark samples ranging from 2.22±0.08 (*F. infectoria*) to 11.36±0.46 (*F. retusa*).

Veberic *et al.*, (2008) identified gallic acid, chlorogenic acid and syringic acid in the fruits of *F. carica* and the reported amount of chlorogenic acid was (1.71 mg/100g fresh weight) in the *Miljska figa* cultivar was higher than our determined amounts of chlorogenic acids in the fruits of *F. bengalensis, F. infectoria* and *F. religiosa* but lower than our calculated amounts of chlorogenic acid in the fruits of *F. racemosa* and *F. retusa*. The reported amount of syringic acid (0.104 mg/100 g fresh weight) was much lower than our determined amount of syringic acid in the fruits of *F. bengalensis, F. rascemosa* and *F. infectoria*. However syringic acid was not determined in the fruits of *F. infectoria* and *F. retusa*. Manikandan and Jayakumar (2012) identified and quantified different phenolic acids including protocatechuic acid (2.4 µg/g), gentisic acid (18 µg/g), vanillic acid (11.6 µg/g), sringic acid (14.4 µg/g), ferulic acid (16.5 µg/g), caffeic acid (11.2 µg/g) and p-Coumaric acid (4.5 µg/g) in the leaves of *F. bengalensis* and all these reported values are lower than our calculated amounts of above mentioned phenolic acids in the leaves of *F. bengalensis*. Manikandan and Jayakumar (2012) also identified and quantified gentisic acid (9.9 µg/g), p-coumaric acid (4.5 µg/g) and ferulic acid (4.6 µg/g) in the bark of *F. bengalensis*. The reported value of gentisic acid was higher than our calculated value of gentisic acid in the bark of *F. bengalensis* but the reported value of p-coumaric acid was samller than calculated amount of p-coumaric acid in present study and ferulic acid was not identified in the bark sample of *F. bengalensis* investigated in the present study.

Variation in the reported results of phenolic acids, flavonoids and other bio active components is common because many factors affect these results. These factors include extraction method, temperature of extraction; polarity of solvent used for extraction, time spent on extraction and even if these experimental parameters are matched then geographical variations, difference in cultivar / variety of the same species, time of harvesting and different climatic conditions can be the cause of variation. Veberic *et al*., (2008) investigated the phenolics in different varities of *F. carica* at different times of harvesting and reported significant variation with respect to time and variety.

4.9. Identification and quantification of flavonoids by RP-HPLC

Flavonoids identified and quantified (mg/100g DW) in the fruit samples of selected species of *Fius* are represented in table 4.25. Rutin was identified in all the fruit samples investigated in the present study in the range of 13.79±0.59 (*F. retusa*) to 54.09±2.17 (*F. racemosa*) and all the amounts of rutin detected in the fruit samples were significantly ($P<0.05$) different from one another. Myricetin quantified in the fruit samples ranged from 20.09±0.99 (*F. bengalensis*) to 78.88±2.94 (*F. retusa*). The quantities of myricetin present in the fruits of *F. retusa* and *F. infectoria* were not significantly ($P<0.05$) different from one another but both these quantities and the quantities of myricetin present in other fruit samples were significantly ($P<0.05$) different from one another. Among the fruit samples, significantly ($P<0.05$) higher quantity of quercetin was detected in the fruits of *F. religiosa* (10.91±0.50) followed by *F. racemosa* (7.28±0.32), *F. infectoria* (6.11±0.27) and *F. bengalensis* (4.31±0.19) ≈ *F. retusa* (4.02±0.18). Luteolin was detected only in the fruits of *F. infectoria* (0.94±0.04) and it was not detected in other fruit samples examined in the present study. Kaempferol was not detected in the fruits of *F. infectoria* and *F. retusa* and in other three fruit samples it was detected in significantly ($P<0.05$) different levels ranging from 1.17±0.05 (*F. racemosa*) to 7.5±0.32 (*F. retusa*).

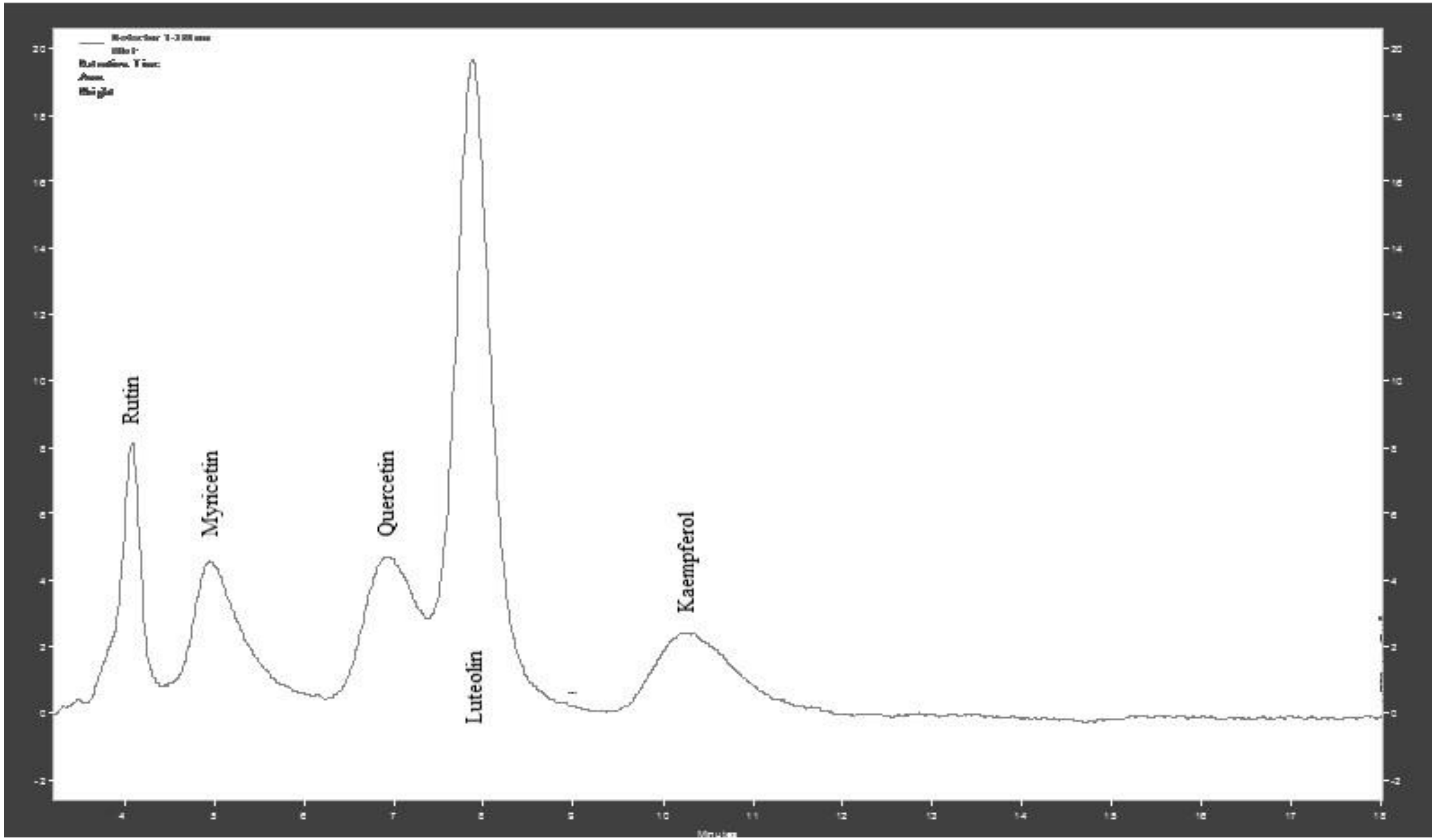

Rutin
Myricetin
Quercetin
Luteolin
Kaempferol

Figure 4.6. HPLC chromatogram, showing separation of flavonoid standards

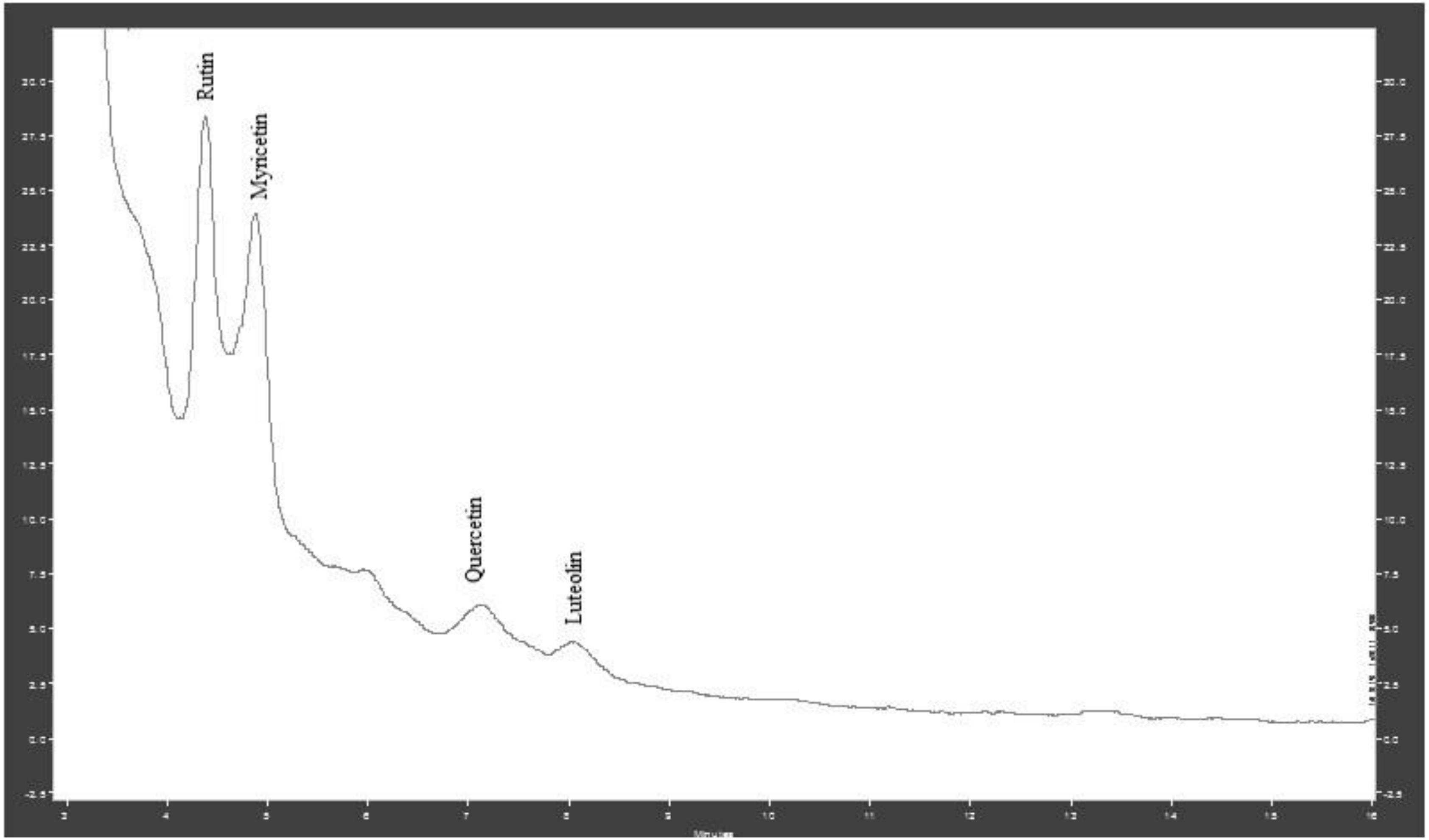


Figure 4.7. A typical chromatogram, showing separation of flavaonoids from the *F.religiosa* leaves extract

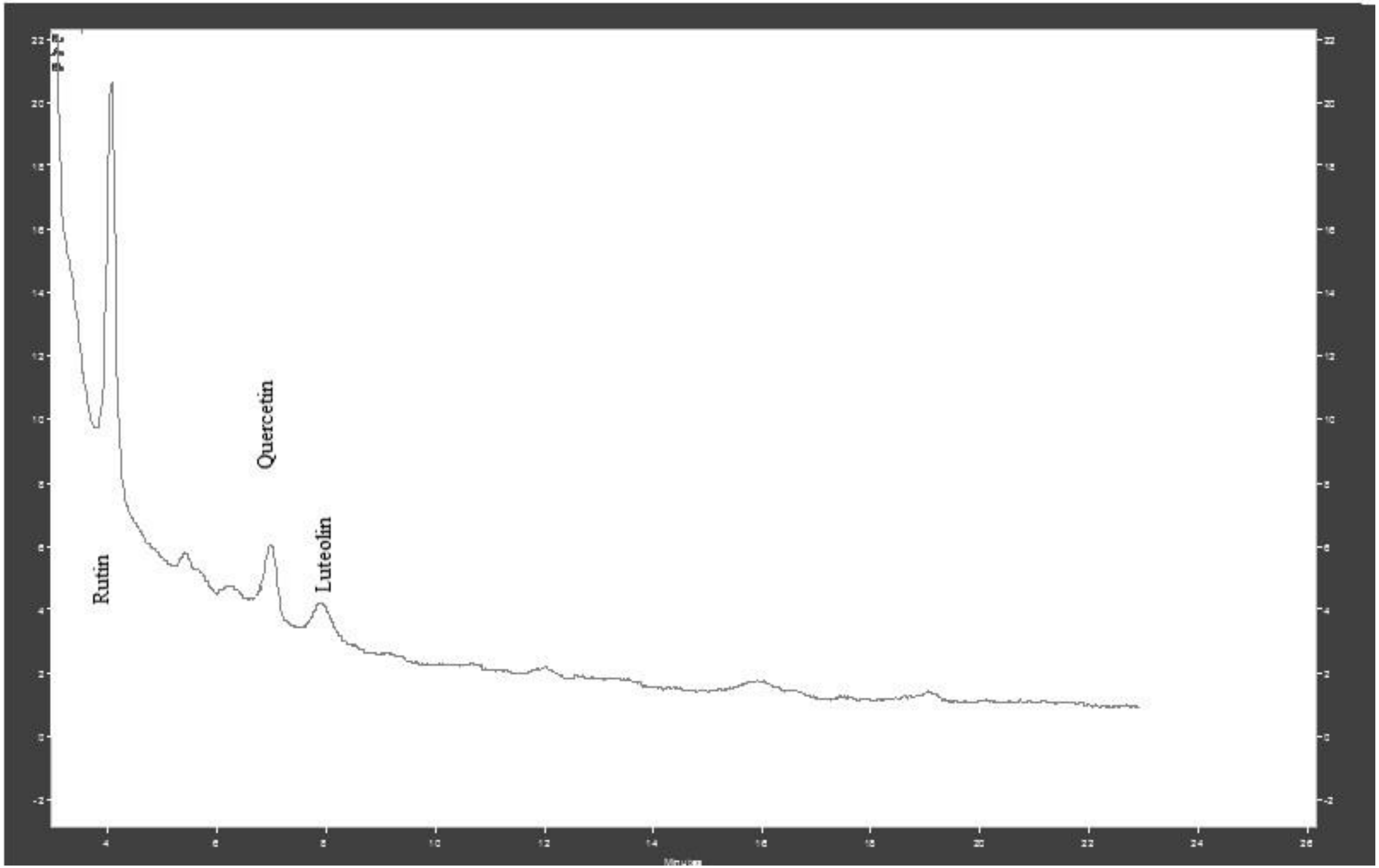


Figure 4.8. A typical chromatogram, showing separation of flavaonoids from the *F.racemosa* bark extract

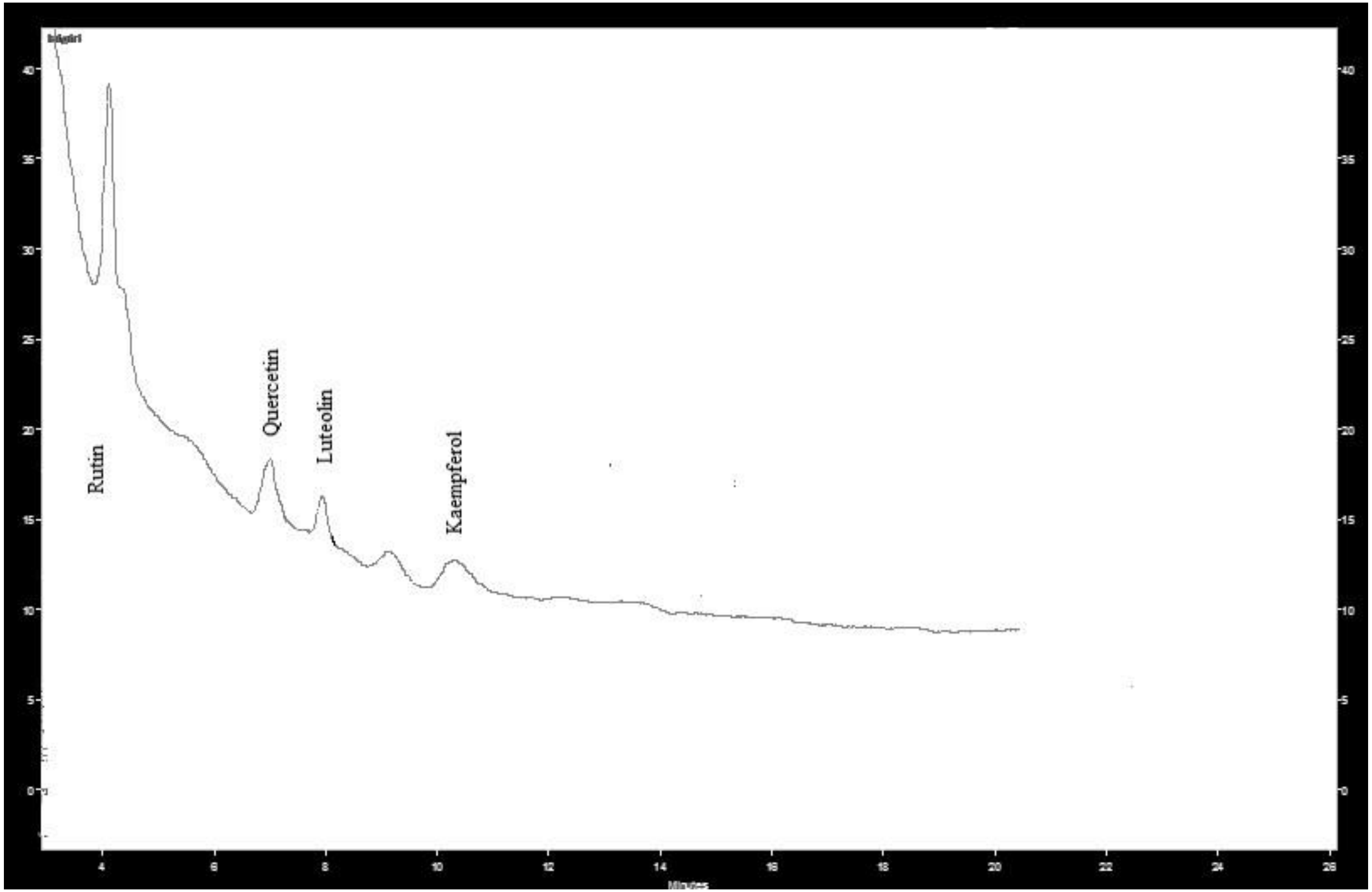


Figure 4. 9. A typical chromatogram, showing separtation of flavaonoids from the *F.religiosa* bark extract Table 4.25. Flavonoid contents (mg/100g of dried sample) quantified by HPLC from the fruits of selected species of *Ficus*

Name	Part	Rutin	Myricetin	Quercetin	Luteolin	Kaempferol
F. bengalensis	Fruit	$16.68\pm0.81_{c}$	$20.09\pm0.99_{c}$	$4.31\pm0.19_{d}$	ND	$2.92\pm0.12_{b}$
F. infectoria	Fruit	$26.40\pm1.23_{b}$	$77.03\pm2.85_{a}$	$6.11\pm0.27_{c}$	$0.94\pm0.04_{a}$	ND
F. racemose	Fruit	$54.09\pm2.17_{a}$	ND	$7.28\pm0.32_{b}$	ND	$1.17\pm0.05_{c}$
F. religosa	Fruit	$16.03\pm0.61_{cd}$	$63.28\pm3.04_{b}$	$10.91\pm0.50_{a}$	ND	$7.5\pm0.32_{a}$
F. retusa	Fruit	$13.79\pm0.59_{d}$	$78.88\pm2.94_{a}$	$4.02\pm0.18_{d}$	ND	ND
LOD		0.03	0.04	0.04	0.01	0.05

*All the values in table are average of three values obtained after the analysis of sample in triplicate and represented as (mean ± SD).
**Subscripts in a column represent different significance levels (p ≤ 0.05) of flavonoid among different species investigated by LSD (least significant difference) test, ***LOD= Limit of detection in mg/Liter

Table 4.26. Flavonoid contents (mg/100g of dried sample) quantified by HPLC from the leaves of selected species of *Ficus*

Name	Part	Rutin	Myricetin	Quercetin	Luteolin	Kaempferol
F. bengalensis	Leaves	$31.81\pm1.05_e$	$37.84\pm1.25_c$	$8.82\pm0.42_b$	$1.31\pm0.05_d$	$1.13\pm0.05_c$
F. infectoria	Leaves	$163.55\pm7.15_b$	$64.56\pm2.18_b$	$15.37\pm0.59_a$	$13.01\pm0.42_a$	$7.32\pm0.31_a$
F. racemose	Leaves	$59.69\pm2.45_d$	$168.95\pm5.88_a$	$0.62\pm0.02_e$	$3.02\pm0.13_b$	ND
F. religosa	Leaves	$80.80\pm3.87_c$	$66.39\pm2.02_b$	$5.52\pm0.22_d$	$1.86\pm0.08_c$	ND
F. retusa	Leaves	$186.67\pm7.93_a$	ND	$7.4\pm0.41_c$	$0.28\pm0.0.01_e$	$1.75\pm0.03_b$
LOD		0.03	0.04	0.04	0.01	0.05

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column represent different significance levels ($p \leq 0.05$) of flavonoid among different species investigated by LSD (least significant difference) test, ***LOD= Limit of detection in mg/Liter

Table 4.27. Flavonoid contents (mg/100g of dry sample) quantified by HPLC from the bark of selected species of *Ficus*

Name	Part	Rutin	Myricetin	Quercetin	Luteolin	Kaempferol
F. bengalensis	Bark	$31.08\pm0.86_d$	ND	$3.57\pm0.17_b$	$0.32\pm0.07_c$	ND

F. infectoria	Bark	$85.51 \pm 3.55_{b}$	ND	$2.52 \pm 0.11_{c}$	ND	ND
F. racemose	Bark	$61.67 \pm 2.88_{c}$	ND	$1.09 \pm 0.04_{d}$	$0.26 \pm 0.01_{c}$	ND
F. religosa	Bark	$96.19 \pm 3.45_{a}$	ND	$9.1 \pm 0.45_{a}$	$4.7 \pm 0.23_{a}$	$1.96 \pm 0.09_{a}$
F. retusa	Bark	$31.54 \pm 1.42_{d}$	ND	$2.76 \pm 0.11_{c}$	$0.56 \pm 0.02_{b}$	ND
LOD		0.03	0.04	0.04	0.01	0.05

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column represent different significance levels ($p \leq 0.05$) of flavonoid among different species investigated by LSD (least significant difference) test, ***LOD= Limit of detection in mg/Liter

The results obtained after the RP-HPLC of the leaf samples of selected species of *Ficus* are tabulated in table 4.26. Fairly good amounts of flavonoids were detected in all the leaf samples investigated in the present study. Rutin was determined in all the leaf samples studied and significantly (P<0.05) higher amount of rutin was detected in the leaves of *F. retusa* (186.67±7.93) followed by *F. infectoria* (163.55±7.15), *F. religiosa* (80.80±3.87), *F. racemosa* (59.69±2.45) and *F. bengalensis* (31.81±1.05). Myricetin was not detected in the leaves of *F. retusa* and in other four leaf samples it was detected in the range of 37.84±1.25 (*F. bengalensis*) to 168.95±5.88 (*F. racemosa*). There was no significant (P<0.05) difference found between the quantities of myricetin found in the leaves of *F. religiosa* (66.39±2.02) and *F. infectoria* (64.56±2.18). Quercetine was found in all the leaf samples tested in the present study and the ranking of leaf samples on the basis of quercetin contents from higher to lower is *F. infectoria* (15.37±0.59) > *F. bengalensis* (8.82±0.42) > *F. retusa* (7.4±0.41) > *F. religiosa* (5.52±0.22) > *F. racemosa* (0.62±0.02). Luteolin was also found in all the leaf samples ranging from 0.28±0.0.01 (*F. retusa*) to 13.01±0.42 (*F. infectoria*) and the amounts of luteolin quantified in all the leaf samples were significantly (P<0.05) different from each other. Kaempferol was detected only in the leaves of *F. bengalensis*, *F. infectoria* and *F. retusa* in significantly (P<0.05) different amounts in the range of 1.13±0.05 (*F. bengalensis*) to7.32±0.31 (*F. infectoria*).

Flavonoids identified and quantified in the barks of the selected species of *Ficus* are represented in the table 4.27. Rutin was identified in all the bark samples examined in the present study in the range of 31.08±0.86 (*F. bengalensis*) to 96.19±3.45 (*F. religiosa*). There was no significant (P<0.05) difference found between the amounts of rutin detected in the barks of *F. bengalensis* and *F. retusa* although the amounts of rutin found in other bark samples were significantly (P<0.05) different from each other. Myricetin was not detected in any of the bark sample tested in the present study however quercetin was found in all the bark samples of *Ficus* species tested in the present study. Among the bark samples, significantly (P<0.05) higher quantities of quercetin were detected in the bark of *F. religiosa* (9.1±0.45) followed by *F. bengalensis* (3.57±0.17), *F. retusa* (2.76±0.11) ≈ *F. infectoria* (2.52±0.11) and *F. racemosa* (1.09±0.04). Luteolin was not detected in the bark of *F. infectoria* but it was quantified in other four bark samples in the range of 0.26±0.01 (*F. racemosa*) to 4.7±0.23 (*F. religiosa*). The quantities of luteolin found in the barks of *F. racemosa* and *F. bengalensis* were not significantly (P<0.05) different.

Sultana *et al.*, (2008) reported the quantity of different flavonols detected in different parts of medicinal plants. The reported values in the fruit of *F. religiosa* were 694.0 ± 13.9 mg/kg for myricetin, 256.3 ± 2.6 mg/kg for quercetin and 160.8 ± 4.8 mg/kg kaempferol. These reported values are higher than our detected quantities myricetin(63.28±3.04), quercetin (10.91±0.5) and kaempferol (7.5±0.32) in the fruit of *F. religiosa*. The reported value in the leaves of *Moringa oleifera* for myricetin (5804.4 ± 116.1 mg/kg) is much higher even than our calculate highest value 168.95±5.88 mg/100 g for myricetin in the leaves samples of *Ficus* but the reported value for myricetin (1283.5 ± 38.5 mg/kg) in the leaves of *A. barbadensis* was lower than our calculated highest value 168.95±5.88 mg/100 g of in the leaf samples but this reported value is higher than our calculated quantities of myricetin in the leaves of *F. bengalensis, F. infectoria* and *F. religiosa*. The reported quantity of quercetin (281.0 ± 5.6) in the leaves of *Moringa oleifera* was also higher than the values of quercetin in all the leaf samples investigated in present study. The reported value of kaempferol (40.2 ± 0.8mg/kg) in the leaves of *Moringa oleifera* was higher than the calculated values of kaempferol in the leaves of *F. bengalensis* and *F. retusa* but lower than that of *F. infectoria* leaves. But the reported amount of kaempferol in the leaves of *A. barbadensis* (257.7 ± 5.2 mg/kg) was higher than our calculated amount of kaempferol in the leaf samples tested in present study. Sultana *et al.*, (2008) scrutinized the bark of *Eugenia jambolana, Azadirachta indica* and *Terminalia arjuna* for their flavonol contents and reported all of them devoid myricetin which is in agreement with our conclusion about the presence of myricetin in the tested bark samples. Veberic *et al.*, (2008) reported rutin in the fruits of *F. carica* from three different cultivars. The highest amount of rutin (28.7 mg/100 g fresh weight) was reported in the *Miljska figa* cultivar and this reported value is higher than our determined amount of rutin in the fruits of *F. bengalensis, F. infectoria, F. religiosa* and *F. retusa* but the reported value of rutin is lower than our determined value of rutin in the fruits of *F. racemosa*. The reported amount of rutin (68.21 mg/100 g) by (Nakilcioglu *et al.*, 2013) in the fruits of *F. carica* is higher than our calculated quantities of rutin in all the fruit samples investigated in the present study. The reported amounts of rutin (9.55±0.09 mg/g), quercetin (7.75±0.44 mg/g) in methanolic extract of taif rose are much higher than our calculated values of rutin and quercetin for all the samples scrutinised in the present study.

Table 4.28. Antimicrobial activity (Inhibition zone in mm) of the extract from fruits of selected species of *Ficus* using disc diffusion method

		Gram positive			Gram negative			Fungi		
Name	Part	*B. cereus*	*B.subtilis*	*S. aureus*	*E.aerogenes*	*E.coli*	*P.aeruginosa*	*A.niger*	*R.oryzae*	*S. cerevisiae*
F. bengalensis	Fruit	$13.0\pm0.6_a$	9.0 ± 0.37	$8.0\pm0.35_d$	Nil	Nil	$11.2\pm0.52_b$	Nil	Nil	Nil
F. infectoria	Fruit	$11.2\pm0.49_b$	Nil	$13.2\pm0.62_b$	Nil	Nil	$11.2\pm0.53_b$	Nil	Nil	Nil
F. racemosa	Fruit	$9.1\pm0.44_d$	Nil	Nil	Nil	Nil	$10.3\pm0.48_c$	Nil	Nil	Nil
F. religiosa	Fruit	$10.3\pm0.49_c$	Nil	$14.1\pm0.52_a$	Nil	Nil	Nil	Nil	Nil	Nil
F. retusa	Fruit	$9.1\pm0.45_d$	Nil	$11.1\pm0.52_c$	Nil	Nil	$13.4\pm0.62_a$	Nil	Nil	Nil

All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column represent different significance levels ($p \leq 0.05$) of antimicrobial activity among different species against the same microbe investigated by applying one way ANOVA

Table 4.29. Antimicrobial activity (Inhibition zone in mm) of the extract from the leaves of selected species of *Ficus* using disc diffusion method

		Gram positive			Gram negative			Fungi		
Name	Part	*B. cereus*	*B.subtilis*	*S. aureus*	*E.aerogenes*	*E.coli*	*P.aeruginosa*	*A.niger*	*R.oryzae*	*S. cerevisiae*
F. bengalensis	Leaves	$13.3\pm0.58_{d}$	Nil	$9.2\pm0.41_{d}$	Nil	Nil	$8.0\pm0.34_{c}$	Nil	Nil	Nil
F. infectoria	Leaves	$19.1\pm0.82_{b}$	$12.0\pm0.6_{b}$	$11.2\pm0.5_{c}$	$8.0\pm0.33_{b}$	Nil	$10.2\pm0.41_{a}$	Nil	Nil	Nil
F. racemosa	Leaves	$16.2\pm0.79_{c}$	Nil	$15.6\pm0.69_{a}$	Nil	Nil	$9.2\pm0.39_{b}$	Nil	Nil	Nil
F. religosa	Leaves	$11.4\pm0.51_{e}$	$11.3\pm0.53_{c}$	$9.2\pm0.43_{d}$	$8.4\pm0.4_{b}$	Nil	$8.4\pm0.31_{bc}$	Nil	Nil	Nil
F. retusa	Leaves	$22.0\pm0.9_{a}$	$16.1\pm0.63_{a}$	$13.1\pm0.62_{b}$	$12.1\pm0.58_{a}$	Nil	$11.1\pm0.84_{a}$	Nil	Nil	Nil
Reference		32.0 ± 0.8	25.4 ± 0.71	23.6 ± 0.66	26.1 ± 0.96	29.2 ± 0.63	24.3 ± 0.81	22.5 ± 0.82	21.4 ± 0.6	24.1 ± 0.62

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **Subscripts in a column represent different significance levels ($p \leq 0.05$) of antimicrobial activity among different species against the same microbe investigated by applying one way ANOVA

Table 4.30. Minimum inhibitory concentration (MIC) (µg/mL) for strains which were sensitive to the extracts in disk diffusion assay

		Gram positive			Gram negative	
Name	Part	*B. cereus*	*B. subtilis*	*S. aureus*	*E.aerogenes*	*P.aeruginosa*
F. bengalensis	Fruit	500	500	>1000	NA	>1000
F. infectoria	Fruit	500	NA	>1000	NA	>1000
F. racemosa	Fruit	500	NA	NA	NA	>1000
F. religosa	Fruit	500	NA	500	NA	NA
F. retusa	Fruit	250	NA	>1000	NA	>1000
F. bengalensis	Leaves	250	NA	500	NA	>1000
F. infectoria	Leaves	250	500	500	>1000	>1000
F. racemosa	Leaves	500	NA	500	NA	>1000
F. religosa	Leaves	250	500	>1000	>1000	>1000

F. retusa	Leaves	250	500	500	>1000	>1000
Amoxycilline		7.8	7.8	7.8	7.8	7.8

4.10. Antimicrobial activity

Antimicrobial activity for the extracts obtained from the fruits of selected species of *Ficus* is represented in table 4.28. Antimicrobial activity of the extracts from the fruit samples of *Ficus* species tested in the present study against the microbial strains was poor to medium. Bacterial strain *B. cereus* was found to be sensitive against all the fruit extracts of *Ficus* species scrutinised for their antimicrobial activity in the present study. Among the fruit samples, significantly ($p<0.05$) higher inhibitory effect on *B. cereus* was shown by the *F. bengalensis* (13.0±0.61 mm) which was followed by *F. infectoria* (11.2±0.49 mm), *F. religosa* (10.3±0.49 mm) and *F. racemosa* (9.1±0.44 mm) ≈ *F. retusa* (9.1±0.44 mm). *B.subtilis* was only sensitive to the extracts obtained from the fruits of *F. religiosa* and the inhibition zone was (9.0±0.37). *S. aureus* was not sensitive to the extracts obtained from the fruits of *F. racemosa* but it was sensitive to the fruit extracts of other four species. The order of these plants according to their inhibition zone was *F. religosa* (14.1±0.52 mm), *F. infectoria* (13.2±0.62), *F. retusa* (11.1±0.52 mm) and *F. bengalensis* (8.0±0.35 mm) and all these values were significantly ($p<0.05$) different from one another. Among the gram negative bacteria *E.aerogenes* and *E.coli* showed no sensitivity against the extracts obtained from the fruits of *Ficus* species investigated in the present study. *P. aeruginosa* was sensitive to all the fruit samples except the fruits of *F. religosa*. The order of plants according to the significantly ($p<0.05$) different inhibitory levels detected in their fruits against *P. aeruginosa* from higher to lower was F. *retusa* (13.4±0.62 mm)> *F. infectoria* (11.2±0.53 mm) ≈ *F. bengalensis* (11.2±0.52 mm) > *F. racemosa* (10.3±0.48 mm). Fruit extracts of selected species of *Ficus* were investigated against three strains of fungi *A.niger, R.oryzae* and *S. cerevisiae* but none of the strain was found to be sensitive against the extracts obtained from the fruits of *Ficus* species investigated in the present study.

Extracts obtained from the leaves of selected species of *Ficus* exhibited higher inhibitory effects than fruit samples on the microorganisms used to screen their antimicrobial activity. All the leaf extracts of *Ficus* species exhibited the medium to good inhibitory effect against *B. cereus*. Among the leaf samples, significantly ($p<0.05$) higher inhibitory effect against *B. cereus* was demonstrated by the leaves of *F. retusa* (22.0±0.93 mm) which was followed by *F. infectoria* (19.1±0.82 mm), *F. racemosa* (16.2±0.79 mm), *F. bengalensis* (13.3±0.58 mm) and *F. religosa* (11.4±0.51 mm). *B.subtilis* was found resistant against the leaf extracts of *F. bengalensis* and *F. racemosa* but it exhibited significantly ($p<0.05$) different sensitivity to the leaf extracts of *F. retusa* (16.1±0.63

mm), *F. infectoria* (12.0±0.6 mm) and *F. religosa* (11.3±0.53 mm). All the leaf extracts of the selected species of *Ficus* exhibited inhibitory effect against the growth of *S. aureus*. The order of plants according to the different significant ($p<0.05$) levels found in the antimicrobial activity of their leaf extracts against the *S. aureus* was *F. racemosa* (15.6±0.69 mm)> *F. retusa* (13.1±0.62 mm)> *F. infectoria* (11.2±0.5 mm) and *F. bengalensis* (9.2±0.41 mm) ≈ *F. religosa* (9.2±0.43). *E. aerogenes* was found to be resistant against the leaf extracts of *F. bengalensis* and *F. racemosa* but sensitive to the leaf extracts of *F. infectoria, F. religosa* and *F. retusa*. The inhibitory effects (against *E. aerogenes*) of the leaf extracts of *F. religosa* (8.4±0.4 mm) and *F. infectoria* (8.0±0.33 mm) were not significantly different from each other but both these values were significantly ($p<0.05$) lower than that of *F. retusa* (12.1±0.58 mm). *E.coli* was found to resistant against all the leaf extracts of *Ficus* species tested in the present study while *P. aeruginosa* was found sensitive to all the leaf extracts of *Ficus* species scrutinised for their antimicrobial activities in the present study. The order of the plants according to the different significant ($p<0.05$) levels found in the inhibitory potential of their leaf extracts against *P. aeruginosa* was *F. retusa* (11.1±0.84 mm)> *F. infectoria* (10.2±0.41 mm)> *F. racemosa* (9.2±0.39 mm)> *F. religosa* (8.4±0.31 mm)> *F. bengalensis* (8.0±0.34 mm). All the leaf samples of *Ficus* species investigated in the present study was found inactive against the fungal strains.

All the bark samples of *Ficus* species investigated in the present study was found inactive against all the bacterial and fungal strains.

Minimum inhibitory concentration (MIC) of those extracts found active against the microorganisms used to screen their antimicrobial activity was also determined. According to the results, the MIC of all the fruit samples found active against *B. cereus* was equal to 500 µg/ml except for the fruit sample of *F. retusa* whose MIC was equal to 250 µg/ml while MIC of all the active leaf samples for *B. cereus* was equal to 250 µg/ml except for the MIC of leaf sample of *F. racemosa* whose MIC for *B. cereus* was 500 µg/ml. The MIC values for the fruit samples of *F. bengalensis* and for the leaf samples of *F. infectoria, F. religosa* and *F. retusa* against *B. subtilis* were equal to 500 µg/ml. The MIC of the fruit samples active against the *S. aureus* was greater than 1000 µg/ml and was not detected in this study as the highest concentration used was 1000 µg/ml to determine the MIC values. The MIC values of leaf samples for *S. aureus* were equal to 500 µg/ml except for the leaf sample of *F. religosa* whose MIC value was found to be greater than 1000 µg/ml. The MIC values for all the fruit and leaf samples active against gram negative bacteria were found to be greater than1000 µg/ml. Antimicrobial activity of all the plants was lower than that of reference materials used in this study.

Mousa *et al.*, (1994) evaluated the antimicrobial activity of fruits of four *Ficus* species and reported that none of the fruit sample was active against the fungal strains and this conclusion was in agreement with our findings. Jeong *et al.*, (2009) evaluated antimicrobial activity of the leaf samples from *F. carica* against oral bacteria and reported their MIC range from 0.156 to 5 mg/ml and concluded that the tested samples have strong antimicrobial activity against *A. actinomycetemcomitans*, *S. anginosus*, *S. gordonii*, *P. gingivalis*, and *P. intermedia* where MIC range improved o.156 to 0.625 mg/ml. These MIC value are in agreement with that of leaf samples (250 µg/ml to 500 µg/ml) studied presently. The data obtained after screening of the samples under study for their antimicrobial activity revealed that all the samples exhibited lower antimicrobial activity against the gram negative bacteria as compared to their activity against gram positive bacteria and it is a general trend for the antimicrobial activity of botanical sources and reported by many researchers (Anwar *et al.*, 2009; Wendkon *et al.*, 2012). This difference in antimicrobial activity occurred due to different chemical composition of outer membranes of gram positive and gram negative bacteria (Lambert, 2002; Walsh *et al.*, 2003).

4.11. Antiscalant activity

Two mechanisms/tests (conductivity measurement test, SEM examination) were adopted to evaluate the antiscalant activity of the extracts of fruit, leaf and bark samples of selected species of *Ficus*. These assays were selected on the basis of mechanisms adopted by the antiscalants to inhibit the scaling on different type of surfaces (metallic surface or membrane surface in case of reverse osmosis plants). Antiscalants prevent scaling through three basic mechanisms called thresh hold inhibition, crystal modification and dispersion. These three mechanisms have been explained previously in chapter 1 of this dissertation. The above mentioned assays can efficiently explore the mechanism by which antiscalant act to inhibit the scaling. On the basis of higher phenolic contents and higher activities, 80% methanolic extracts obtained by applying sonication assisted extraction technique were investigated for their antiscalant activity. The results obtained are discussed below

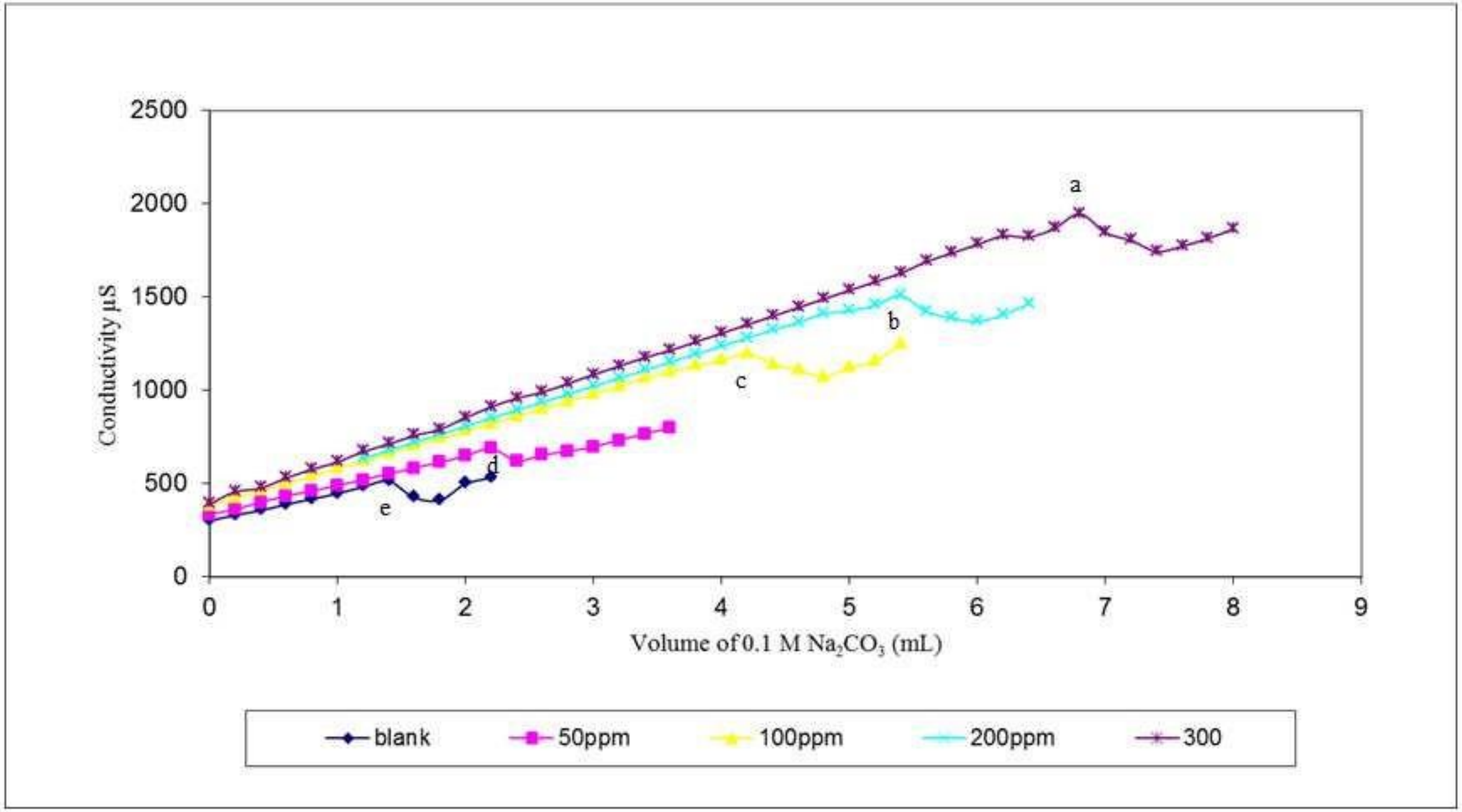


Figure 4.10. Effect of different concentrations of *F. bengalensis* fruit extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

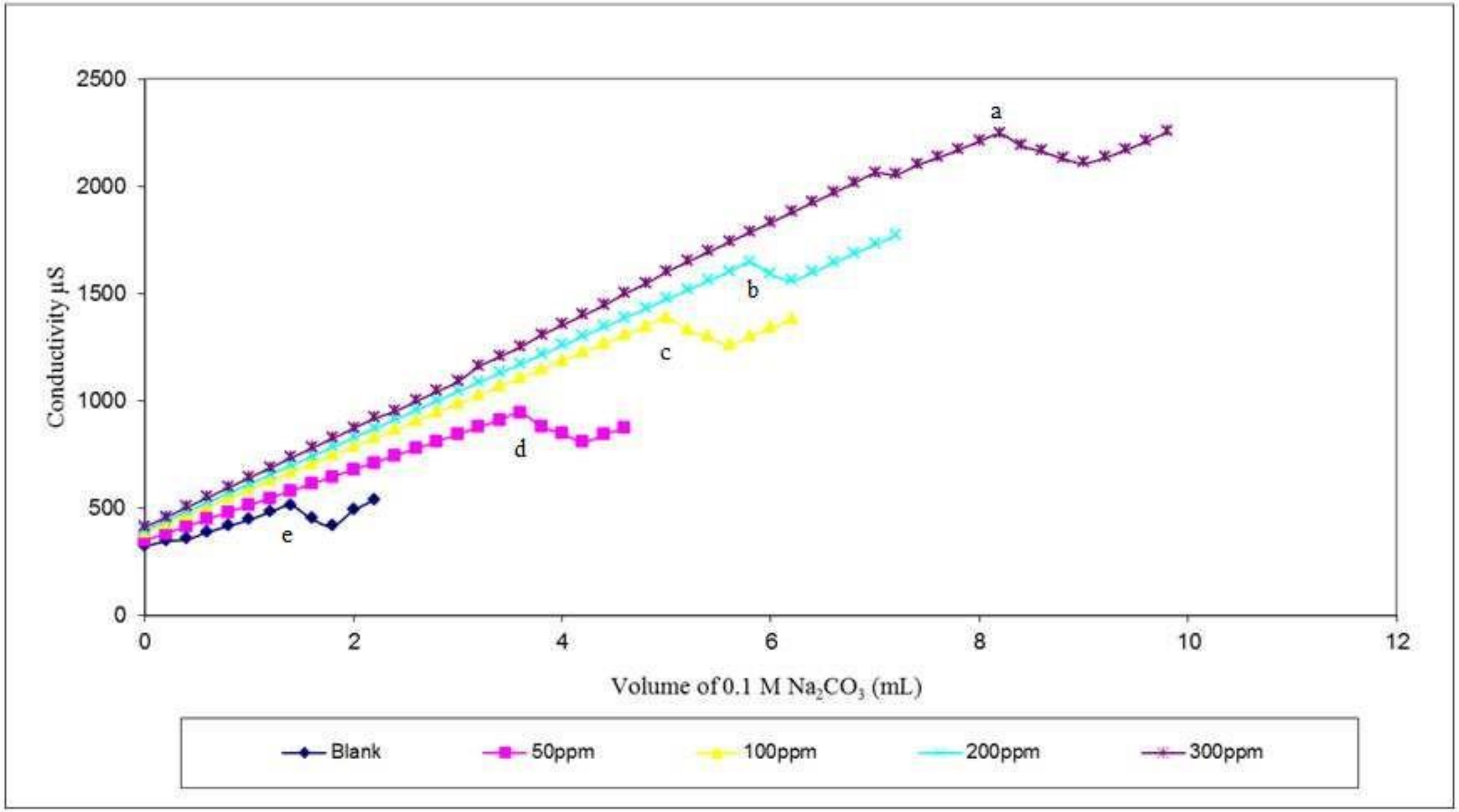


Figure 4.11. Effect of different concentrations of *F. infectoria* fruit extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

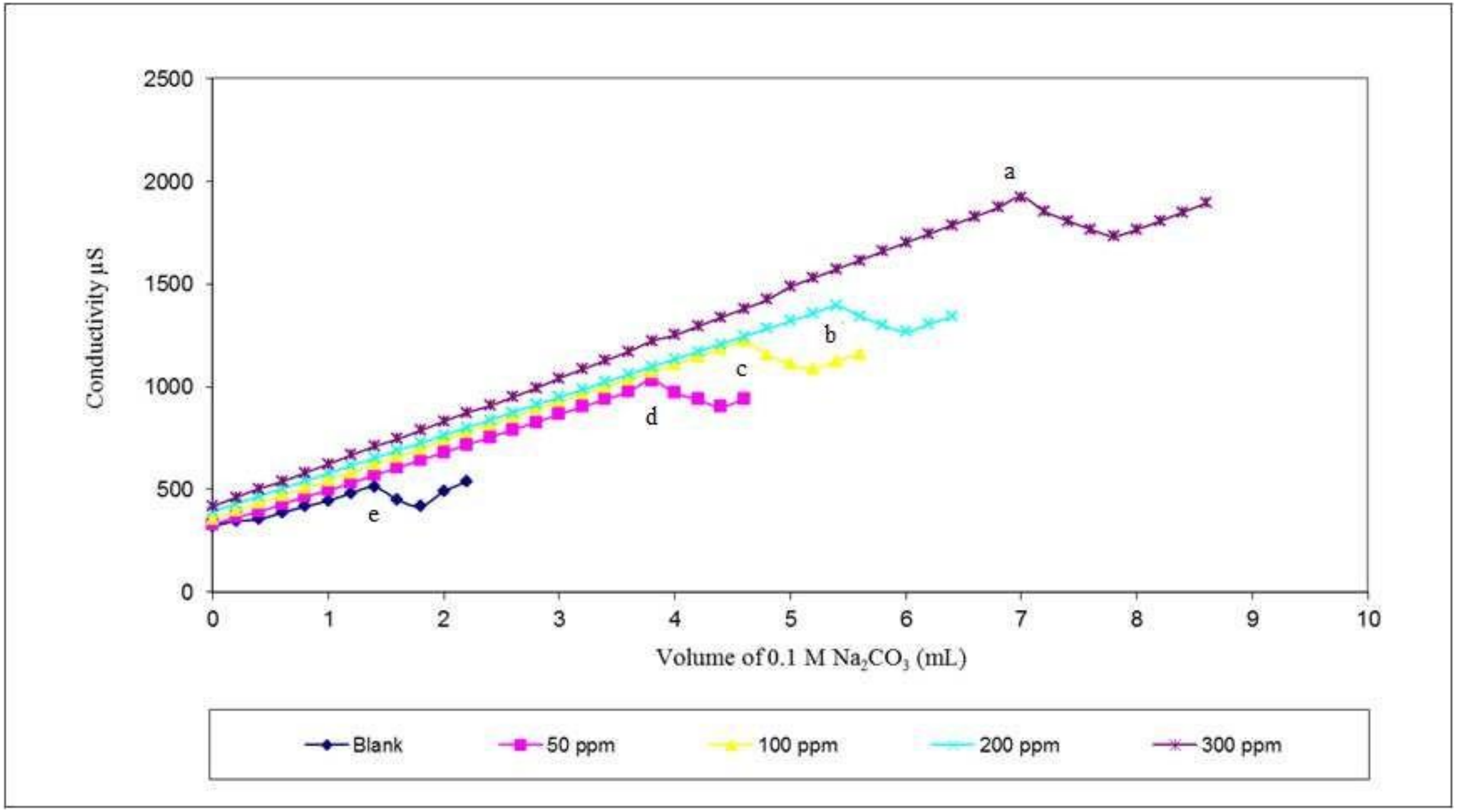


Figure 4.12. Effect of different concentrations of *F. racemosa* fruit extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

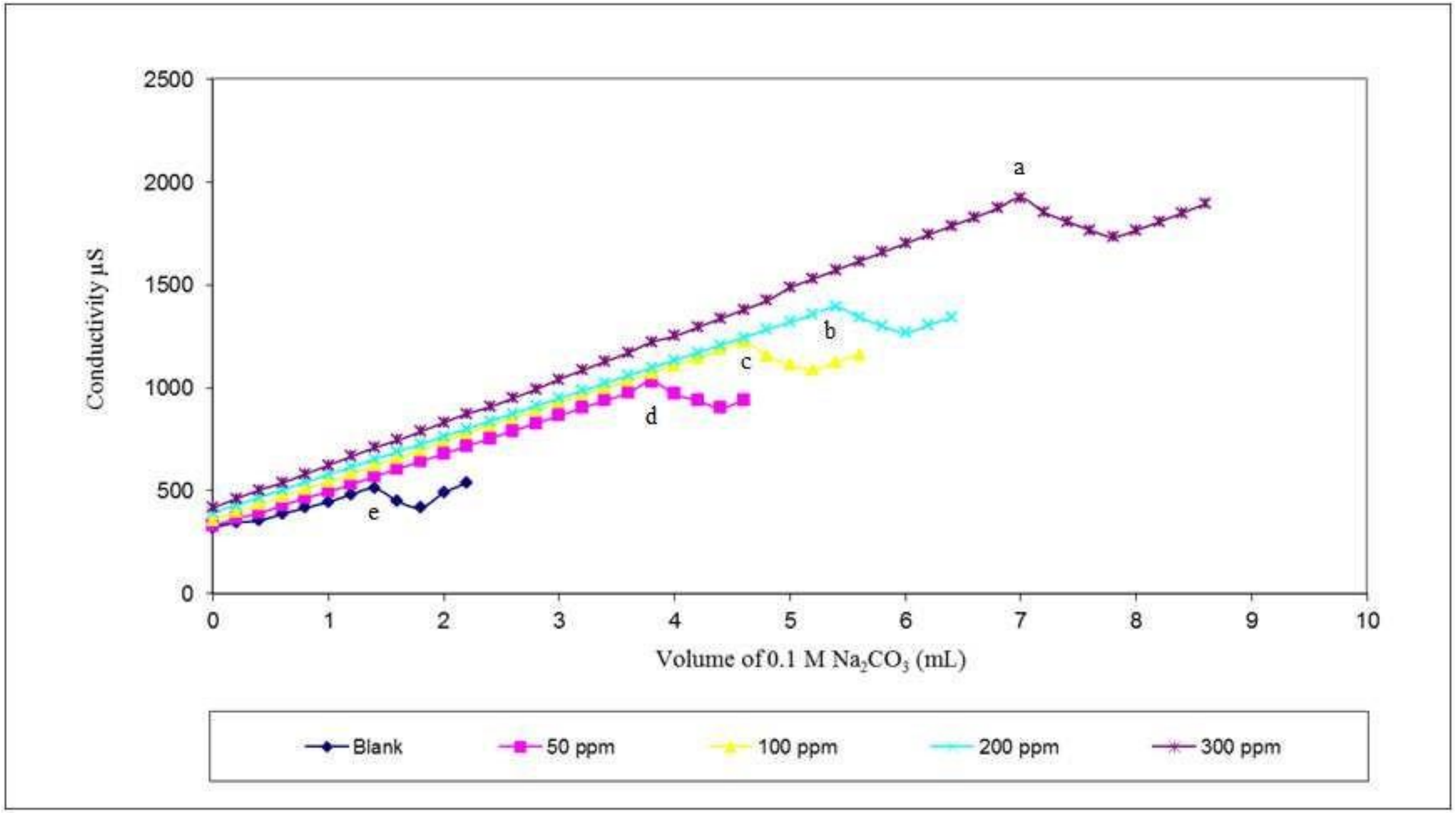


Figure 4.13. Effect of different concentrations of *F. religiosa* fruit extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

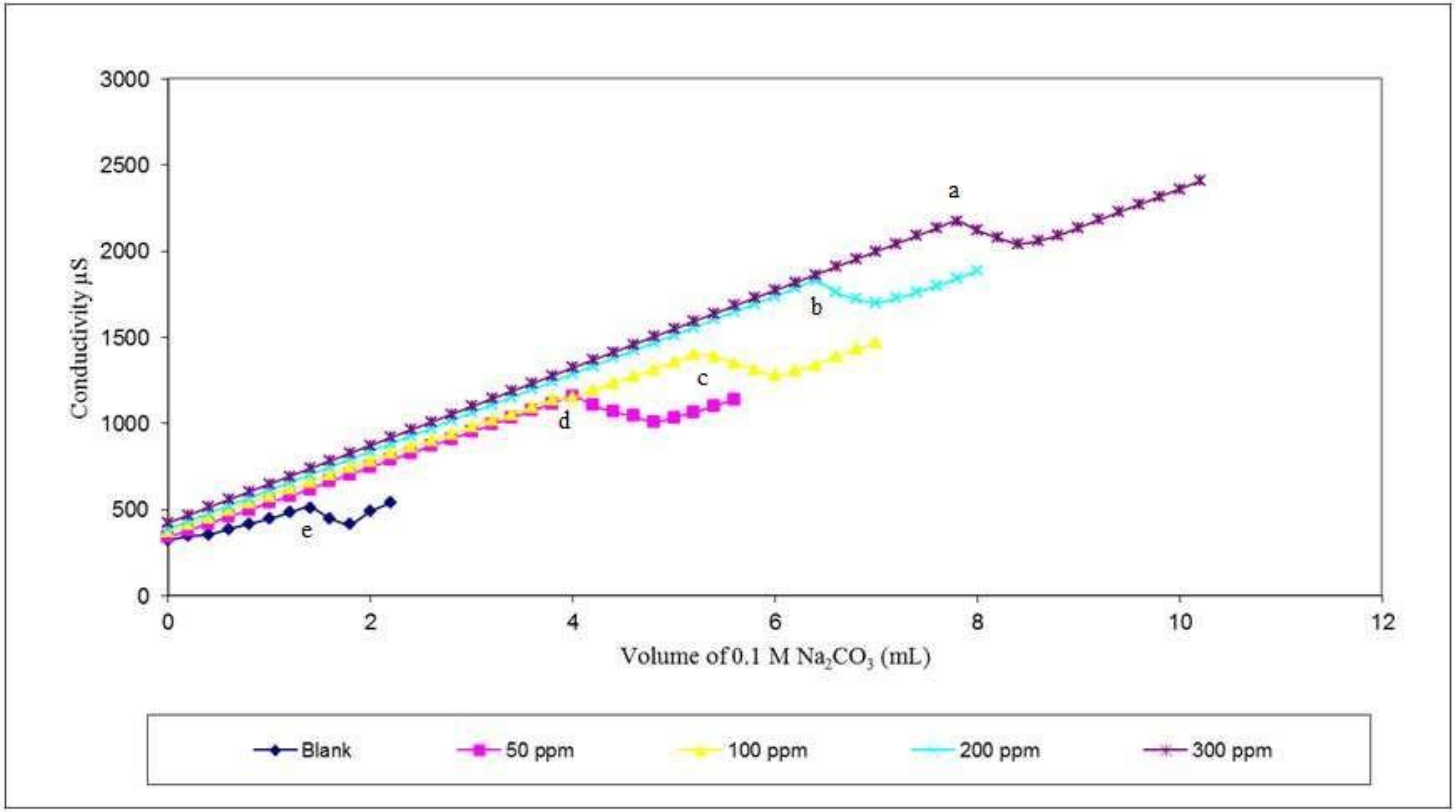


Figure 4.14. Effect of different concentrations of *F. retusa* fruit extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

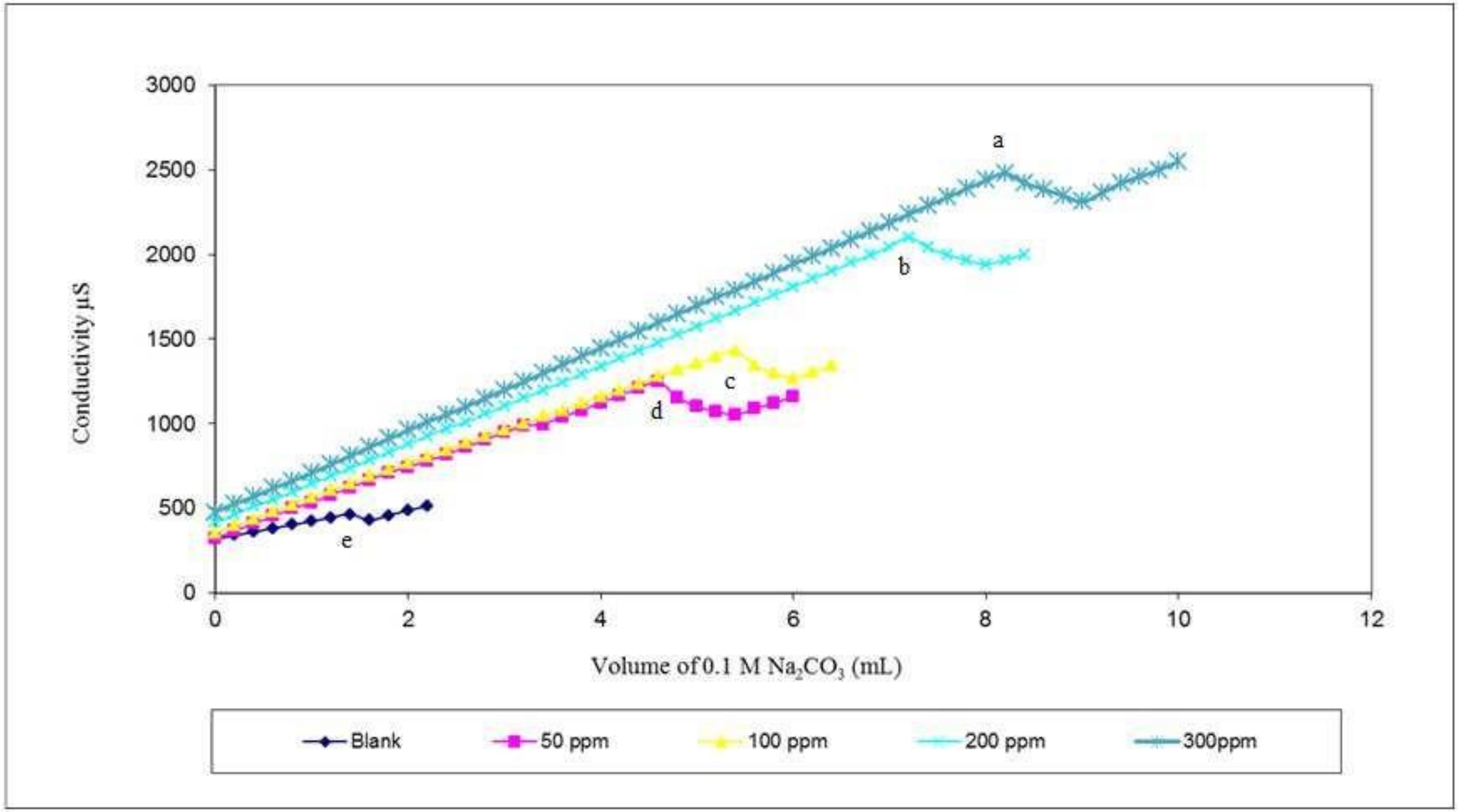


Figure 4.15. Effect of different concentrations of *F. bengalensis* leaves extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

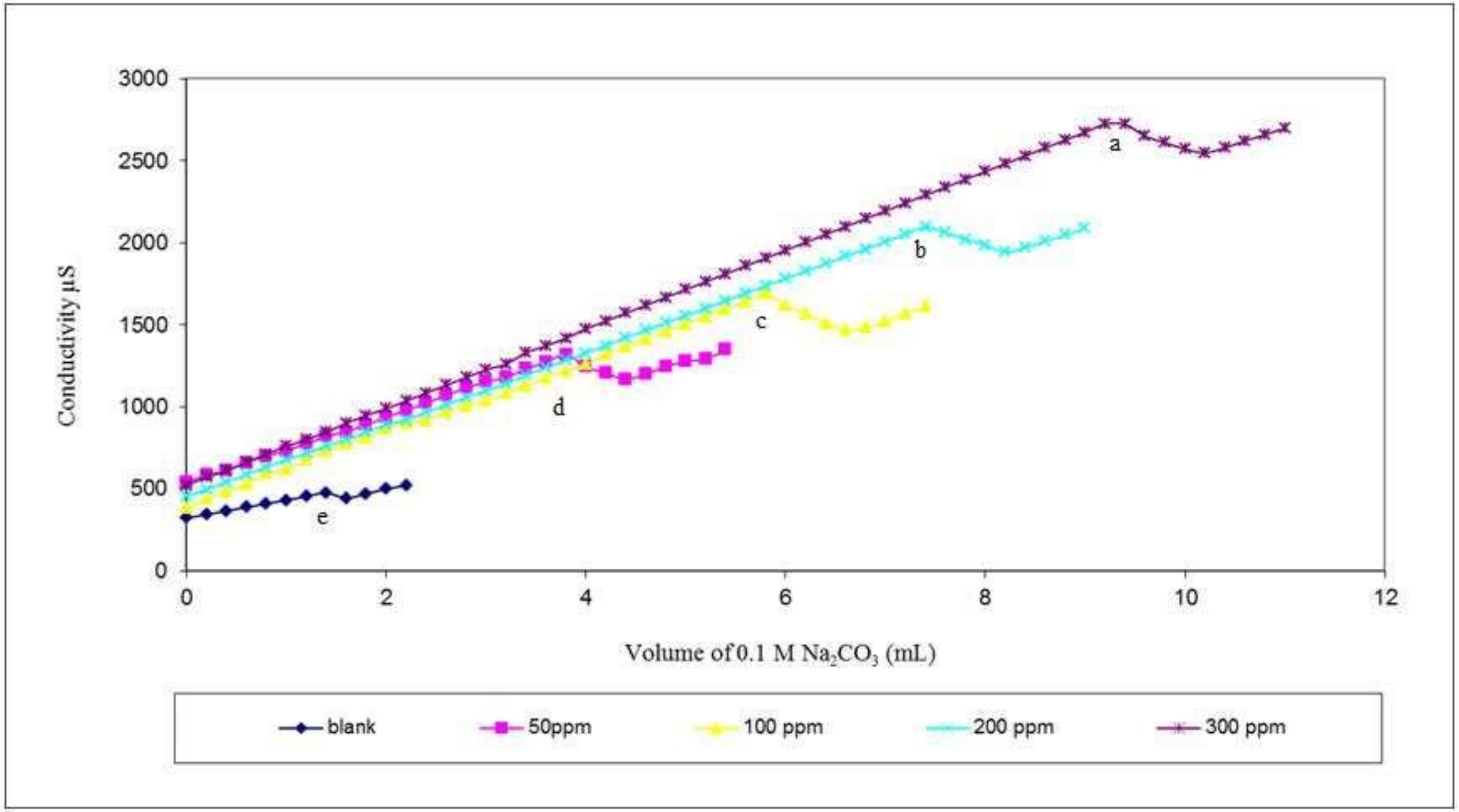


Figure 4.16. Effect of different concentrations of *F. infectoria* leaves extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

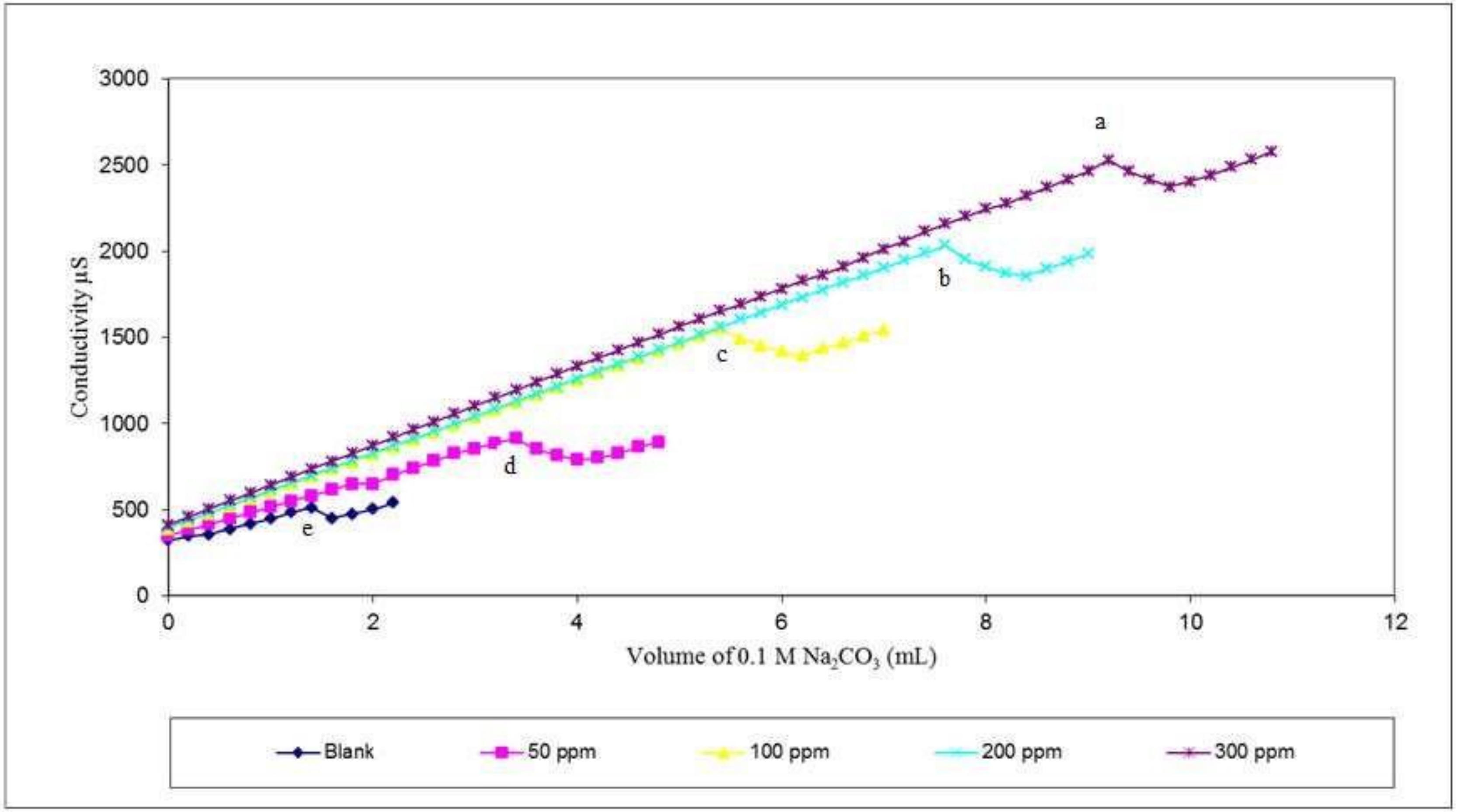


Figure 4.17. Effect of different concentrations of *F. racemosa* leaves extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

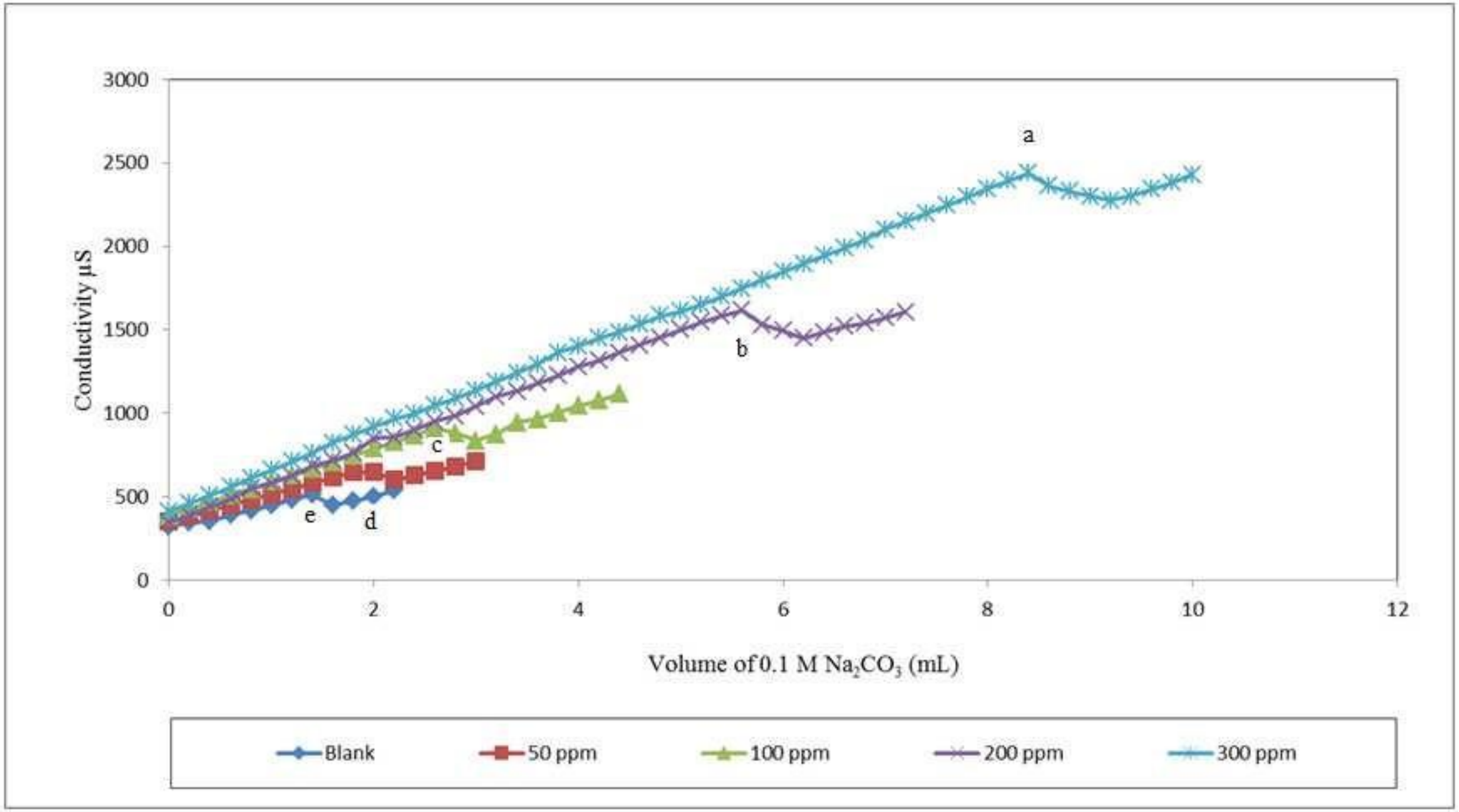


Figure 4.18. Effect of different concentrations of *F. religiosa* leaves extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

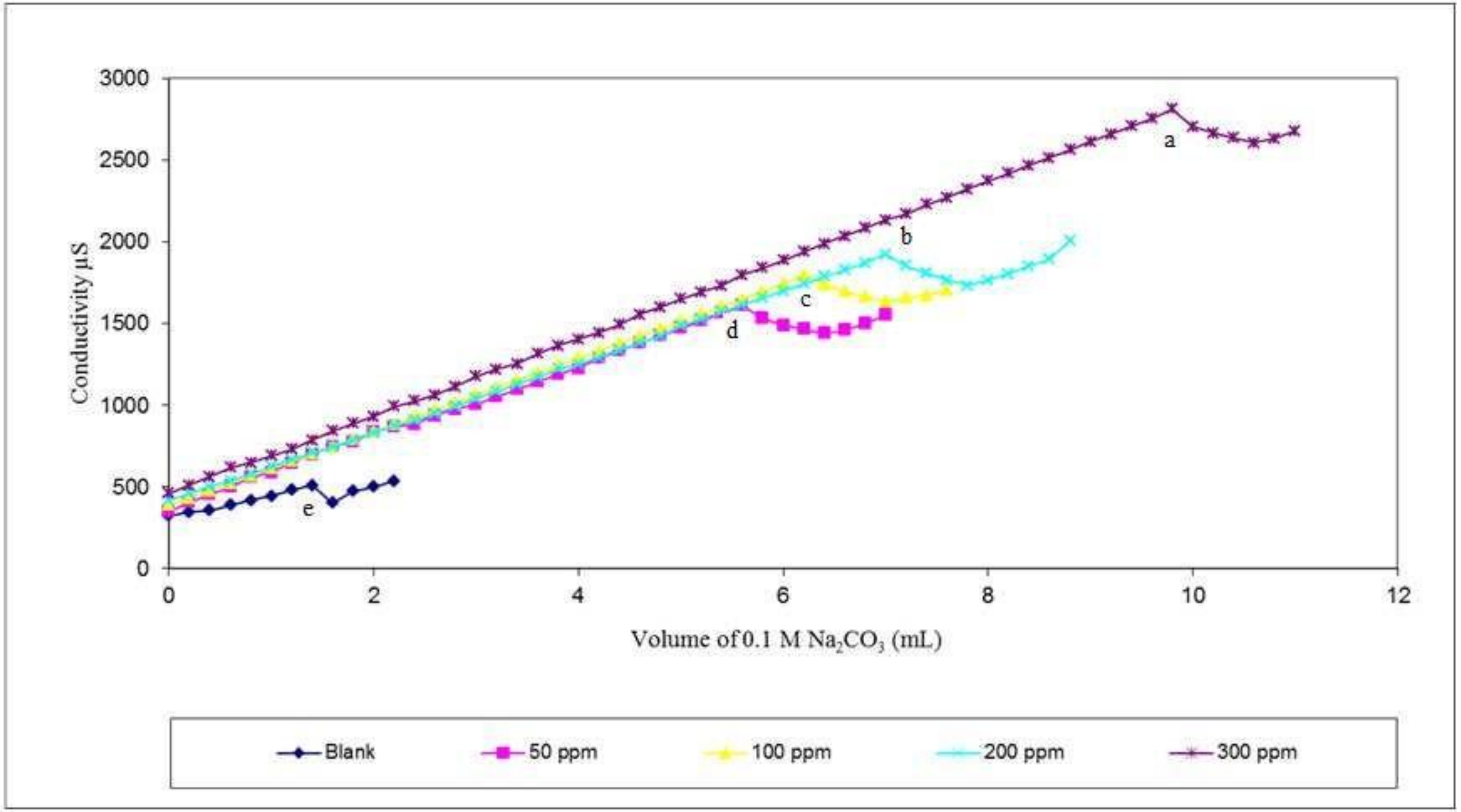


Figure 4.19. Effect of different concentrations of *F. retusa* leaves extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

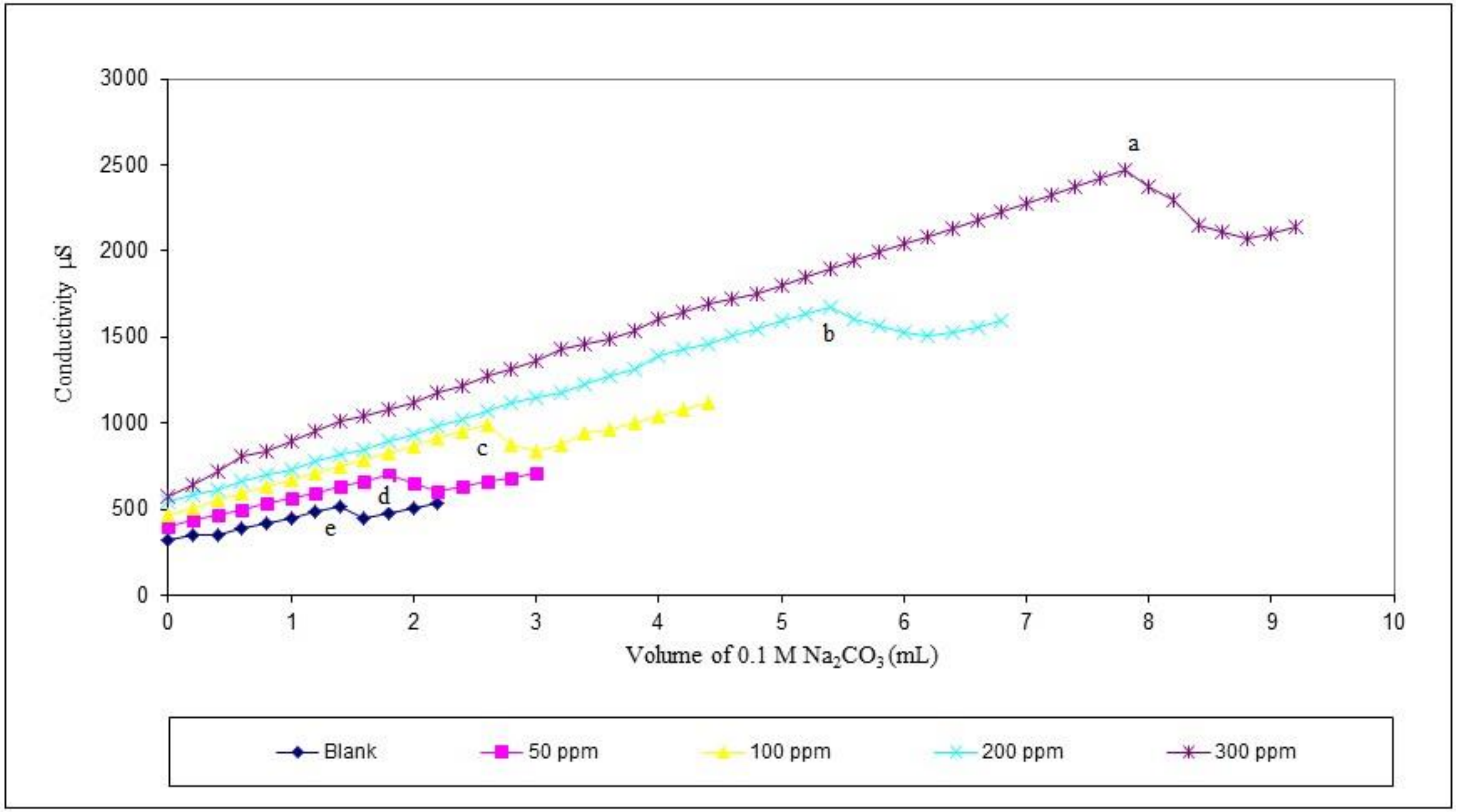


Figure 4.20. Effect of different concentrations of *F. bengalensis* bark extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

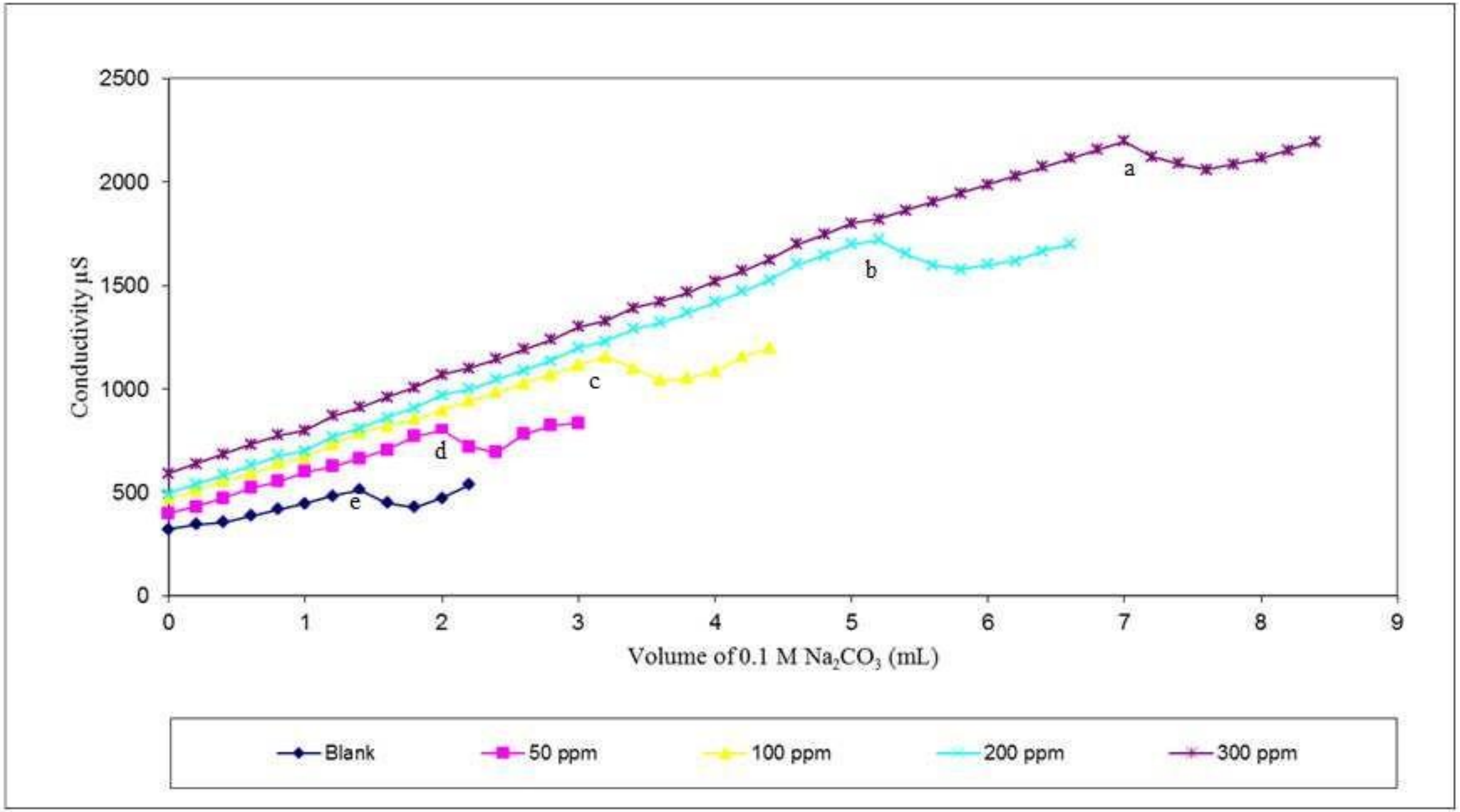


Figure 4.21. Effect of different concentrations of *F. infectoria* bark extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

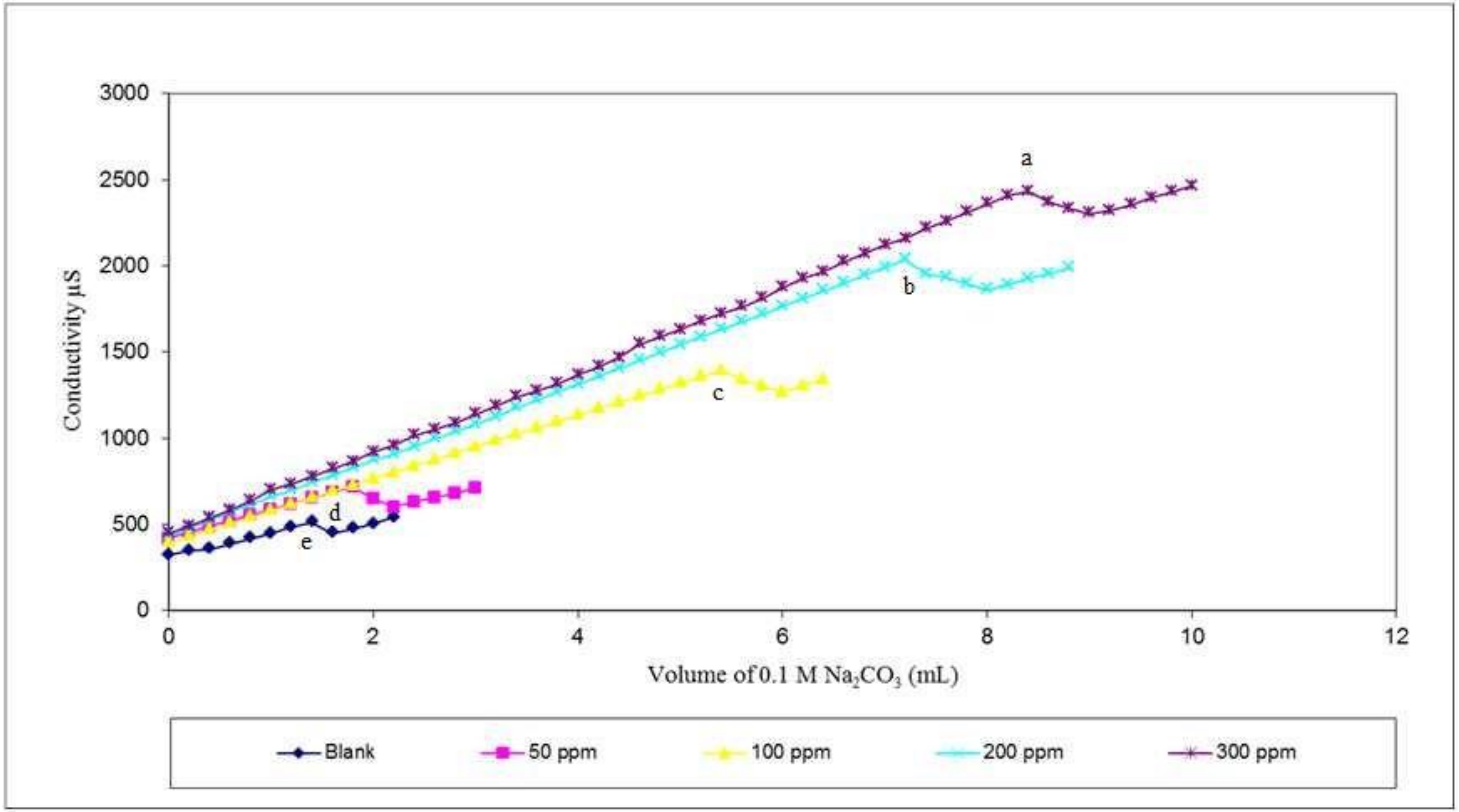


Figure 4.22. Effect of different concentrations of *F. racemosa* bark extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

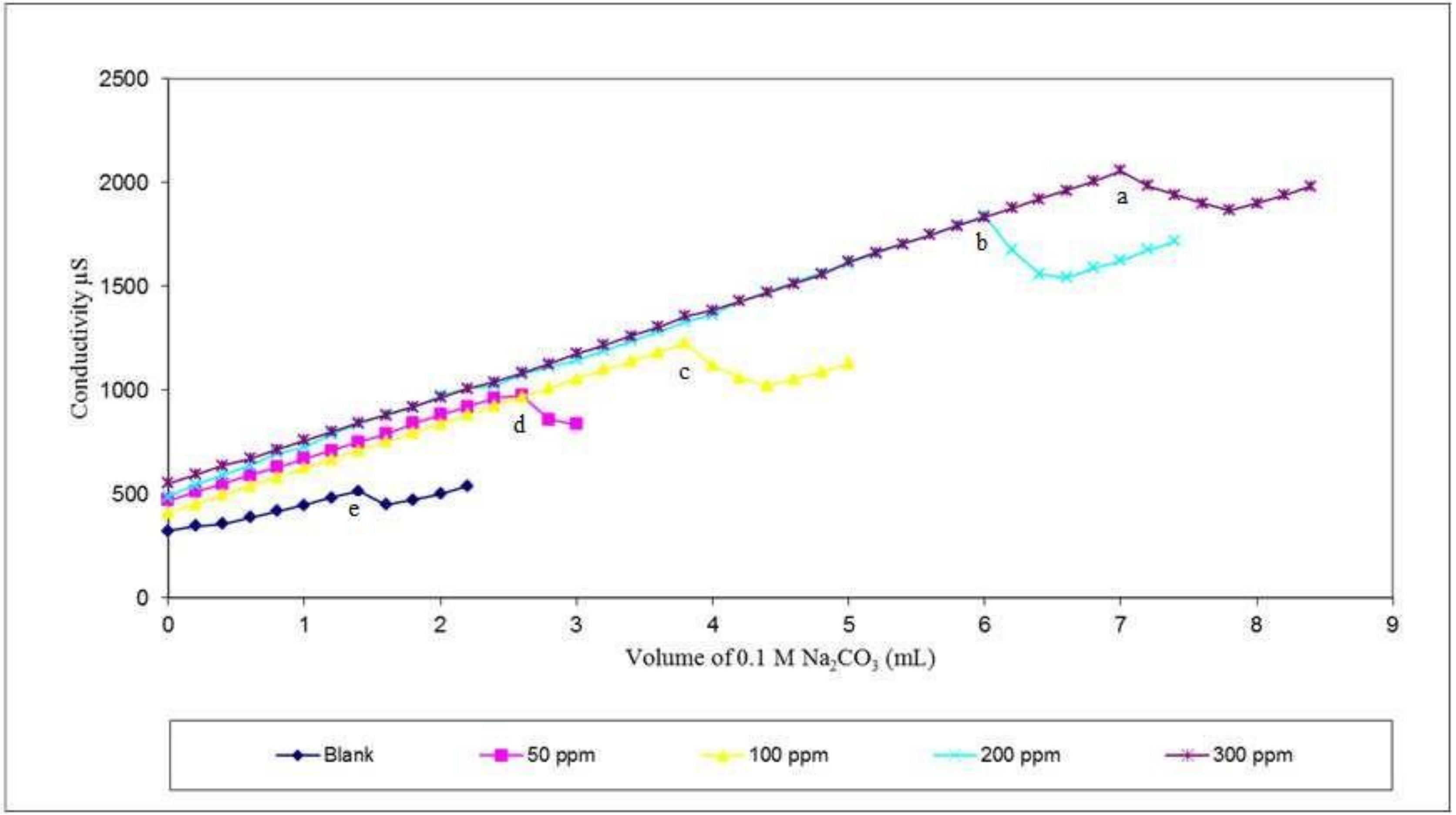


Figure 4.23. Effect of different concentrations of *F. religiosa* bark extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

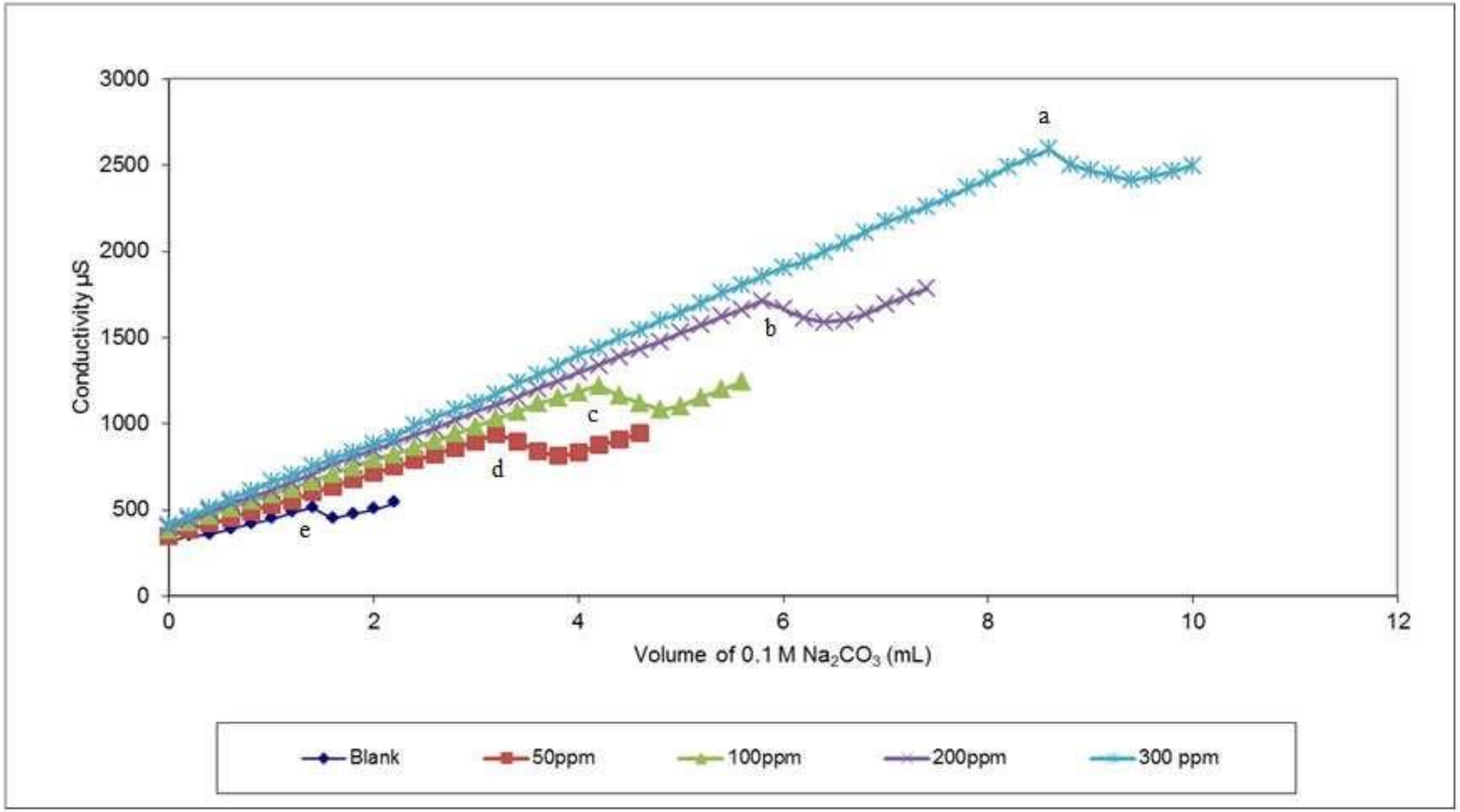


Figure 4.24. Effect of different concentrations of *F. retusa* bark extract on the conductivity of $CaCl_2$ solution with amount of Na_2CO_3 added

4.11.1. Conductivity test

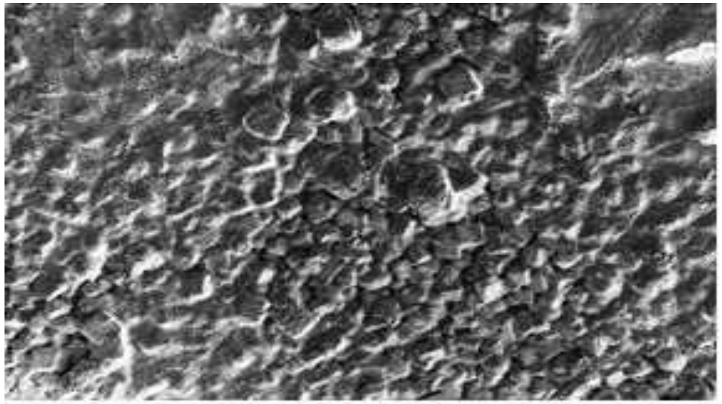
Conductivity test is usually taken as first step to evaluate the antiscalant activity of a compound due to its simplicity, rapidness, reproducibility and being economical. No complex or costly instrument is required just a conductivity meter, beaker and a burette is required to conduct this test. In this test calcium chloride solution is titrated against sodium carbonate solution and conductivity of the solution is measured after each addition. Conductivity of the brine solution increased after the addition of Na_2CO_3 and at a certain point conductivity of the solution decreases suddenly after the addition of Na_2CO_3 and this point is called saturation point. After this point conductivity of the solution again increases after the addition of Na_2CO_3. At the beginning, Na_2CO_3 react with $CaCl_2$ and produce $CaCO_3$ which is sparingly soluble but it remain in the solution in ionic form below its saturation point and the conductivity of the solution increased as the ionic strength of the solution increases by the addition of Na_2CO_3. After a certain level, $CaCO_3$ reaches to its saturation point and precipitated when we add Na_2CO_3. At this saturation point conductivity of the solution decreases as ionic strength of the solution decreases due to precipitation. After this saturation point conductivity of the solution begin to increase again as the ionic strength of the solution increased by the addition of Na_2CO_3. An antiscalant shift this saturation point to higher level and in this way delay the process of scaling and this mechanism is called threshold inhibition. Variation in the conductivity of the $CaCl_2$ in the absence and in the presence of various extracts obtained from the fruits, leave and barks of selected species of *Ficus* were examined at various concentration levels (50ppm, 100ppm, 200ppm and 300ppm). The effect of concentration of the extract on the saturation point was observed to evaluate the antiscalant activity of the extract and graphical presentations of these observations are represented from figure 4.7 to figure 4.21. The observation of results for extracts tested at various concentrations revealed that conductivity of the solutions increased linearly and the saturation point shifts to the higher level. Statistical analysis of saturation points at different concentrations (blank, 50ppm, 100ppm, 200ppm and 300ppm) of the same sample reveled that these saturation points were significantly ($p<0.05$) different from one another and it was true for all the samples studied. The saturation point of $CaCl_2$ solution was equal to (1.4, 552) when no extract was added and saturation point moved to (6.8, 1947) when the fruit extract of *F. bengalensis* was added to the $CaCl_2$ solution and its concentration was 300 ppm. Saturation points at the concentration of 300ppm for various extracts were (6, 1792) for the fruit extract of

F. infectoria, (8.2, 2412) for the fruit extract of *F. racemosa*, (7, 2118) for the fruit extract of *F. religiosa,* (7.8, 2173) for fruit extract of *F. retusa* (8.2, 2478) for leaves extract of *F. bengalensis,* (9.4, 2722) for leaves extract of *F. infectoria*, (9.2, 2712) for leaves extract of *F. racemosa*, (8.4, 2562) for leaves extract of *F. religiosa*, (9.8, 3237) for leaves extract of *F. retusa,* (8, 2375) for bark extract of *F. religiosa*, (7, 2243) for bark extract of *F. infectoria*, (8.4, 2593) for bark extract of *F. racemosa*, (7.6, 2393) for bark extract of *F. religiosa* and (8.8, 2839) for bark extract of *F. retusa.* This data clearly indicate that extracts obtained from different parts of the selected species of *Ficus* shifted the saturation point to the higher level and retain the different ions in the solution for a longer time. In fact the extract obtained from different parts of *Ficus* species is a mixture of different complex molecules including phenolics and flavonoids. These molecules have the ability to release the hydrogen ion in the solution and produce phenolate anion and these anions can react with metal cations including Ca^{+2} in different manners to delay the process of scaling. They can interact with positive sites of growing crystals and inhibit the crystal growth, they can make complex with positive ions like Ca^{+2} and can inhibit its precipitation. These inter actions can occur through different types of chemical forces including Vander Waal's forces, electrostatic force of attraction between positive and negative charges, interaction of unshared electrons present on the extract molecules with active sites of growing particles (Abdel-Gaber *et al.*, 2008). The data obtained by the study of variation in conductivity also revealed that leaf extracts of selected species of *Ficus* are more efficient in shifting the saturation point to higher side as compared to the extracts of fruit and bark.

4.11.2. Scanning electron microscopic examination of scales as affected by extracts

The surface of the steel plates used as cathode was examined by scanning electron microscope. Through this examination we observed the area covered by scale formed in the absence of extracts and in the presence of various concentrations (25ppm, 50ppm, 100ppm) of extracts and in this way we examined the effect of concentration of extract on the scale deposition on the surface of steel plate at electrode potential of -1.2. Typical micrographs are given in chapter 4 from figure 4.22 to 4.36 which explore the effect of extract concentration on the pattern of scale formation. From all these micrographs we concluded that in the absence of extract obtained from different parts of selected species of *Ficus*, surface of the steel plate was covered completely by thick and hard scale film which was hard to remove by mechanical means. But by adding the extracts the pattern of scale formation was changed and hard film of scale was converted to soft one which can be removed easily by mechanical means. From this observation we concluded that presence of extract changed nature of scale from adherent to non-adherent. The second conclusion we made after the observation of these micrographs also revealed that by increasing the concentration, the formation of big crystals of scale was inhibited and smooth film of scale containing tiny particles of scale was formed which can be removed easily by mechanical scratching. From this we can conclude that molecules present in the extract inhibit the nucleation and in this way formation of big crystals was stopped. Abdel-Gaber *et al*., (2012) conducted a study on the antiscalant activity of the extracts obtained from the leaves and hull of *p.grantum* and concluded the similar results as in present study after performing conductivity test and optical microscopic examination.

The results obtained from the conductivity test and microscopic examination clearly indicated that presence of extracts obtained from different parts of *Ficus* species inhibited the process of scale formation through different mechanisms (thresh hold inhibition, dispersion) and made them strong candidate to use them as antiscalant but after further investigations in real situations. Although our investigation and previous reports showed that extracts obtained from different parts of *Ficus* species are rich in phenolic compounds but at this stage it is not fair to credit the antiscalent activity to the presence of phenolics compounds. For this phenolic compounds must be investigated for their antiscalant activities individually.



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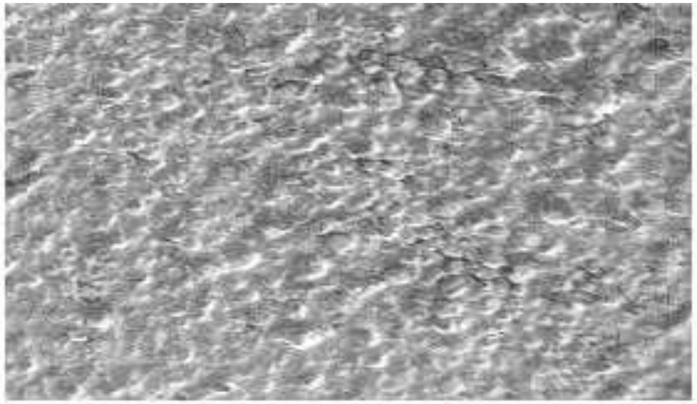

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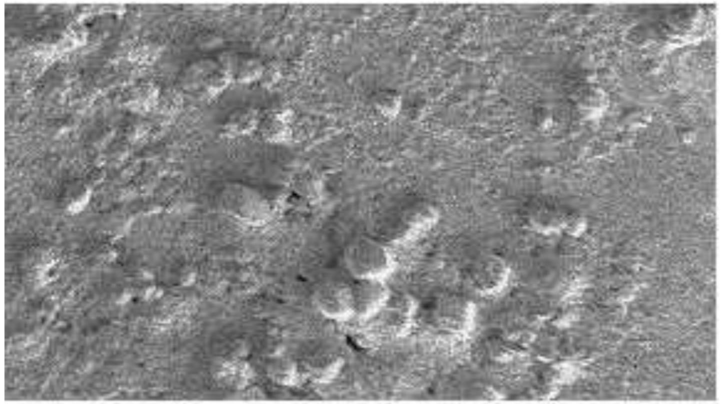
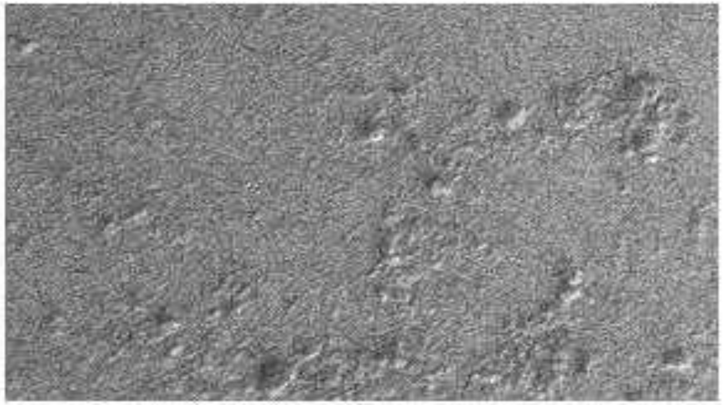
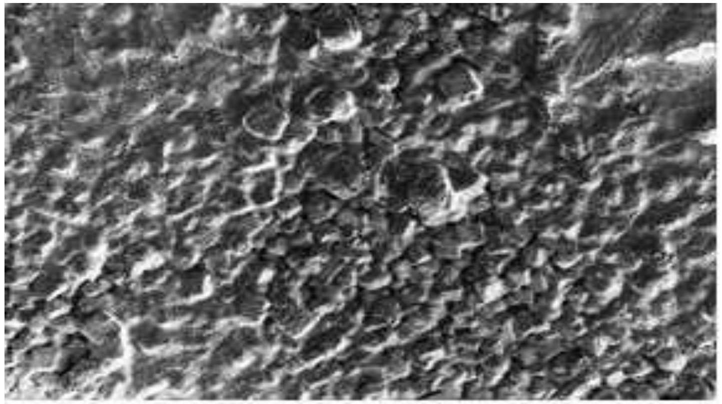


50 ppm, 50X



100 ppm, 50X

Figure 4.25. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the fruits of *F. bengalensis* on scaling



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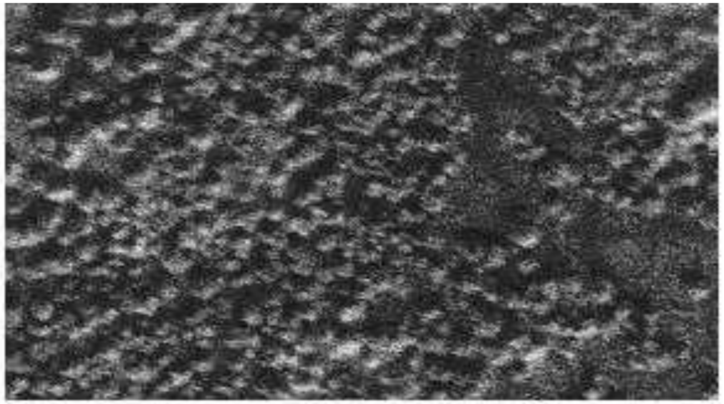

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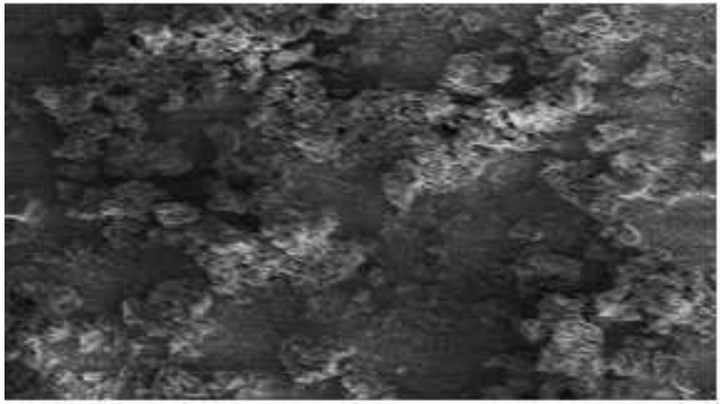

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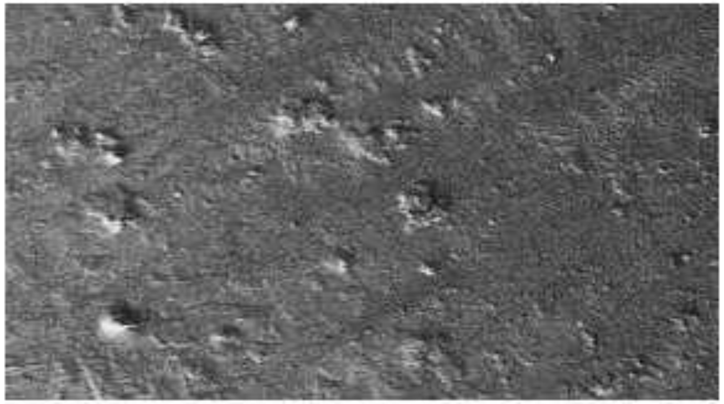
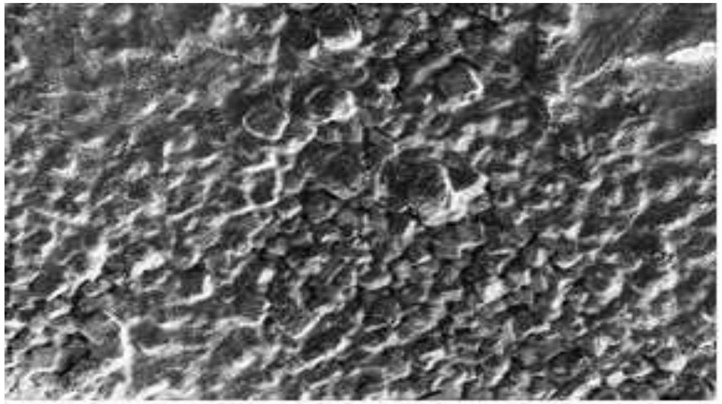


100 ppm, 50X

Figure 4.26. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the fruit of *F. infectoria* on scaling



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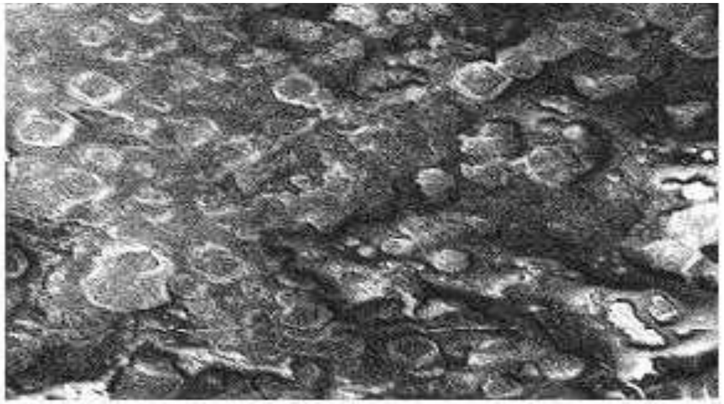

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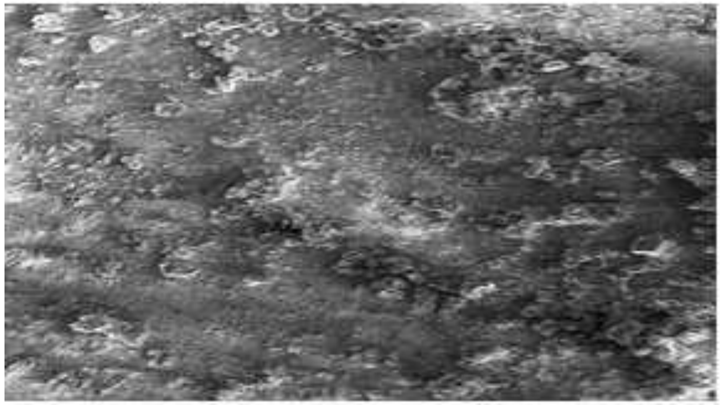

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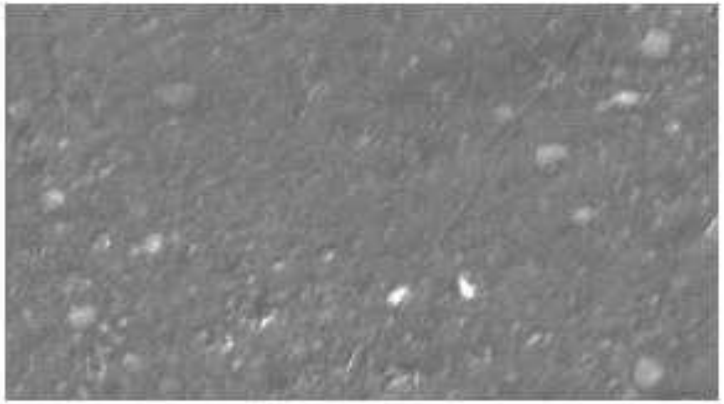
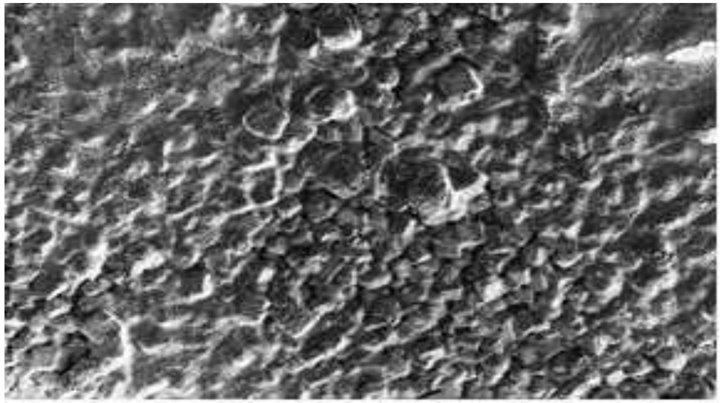


100 ppm, 50X

Figure 4.27. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the fruit of *F. racemosa* on scaling



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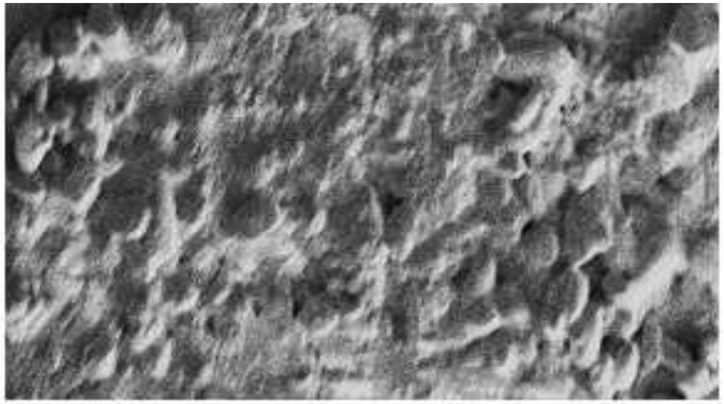

25 ppm, 50X

50 ppm, 50X

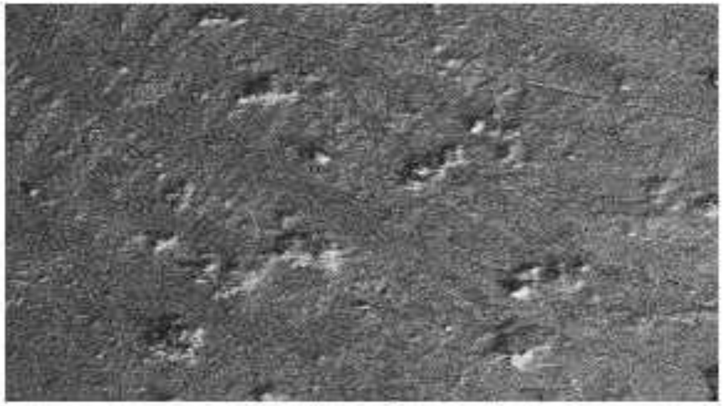

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Figure 4.28. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the fruit of *F. religiosa* on scaling

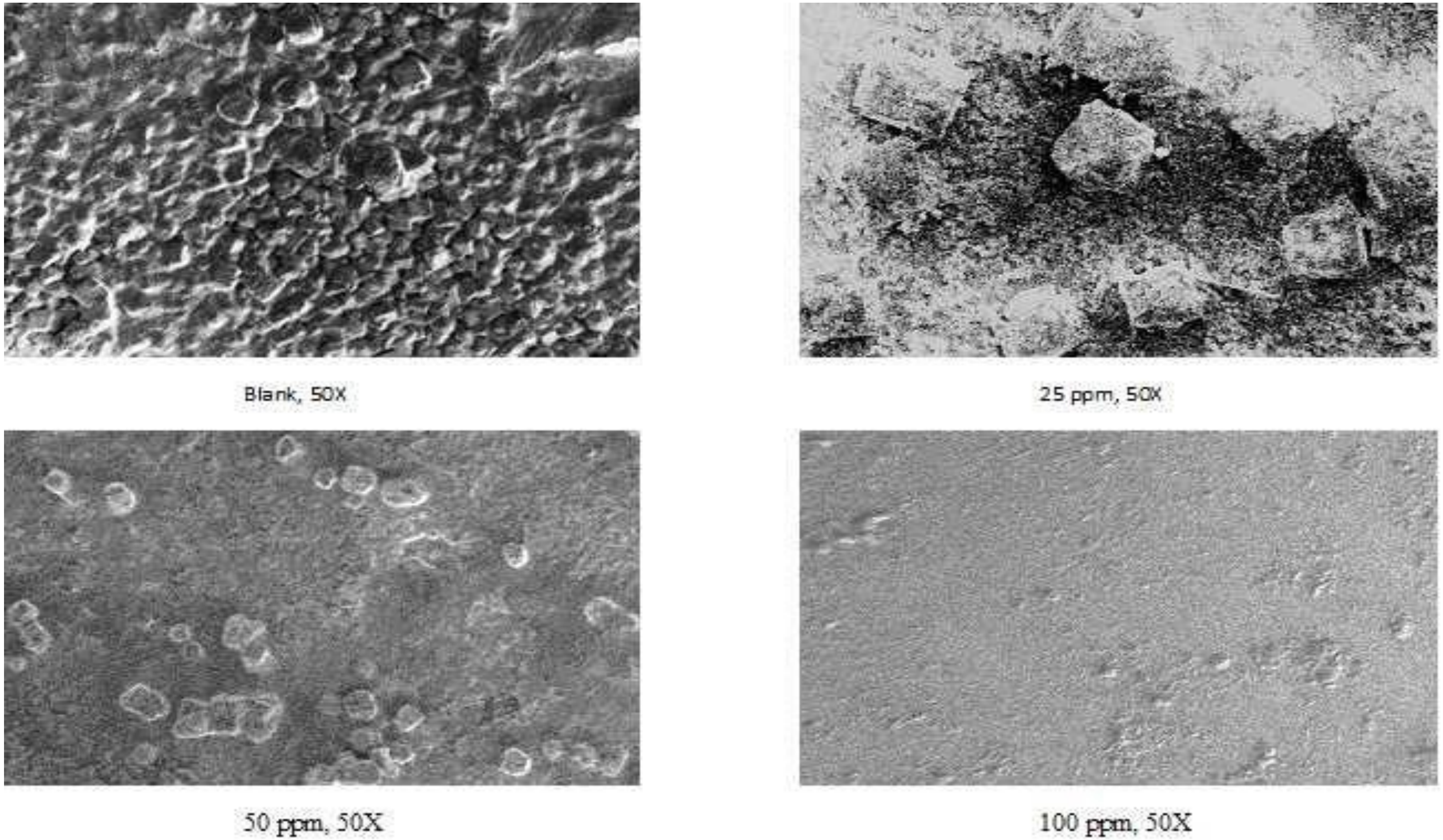
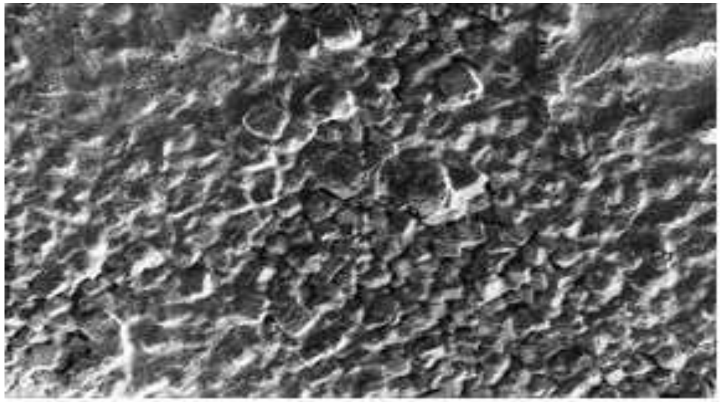


Figure 4.29. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the fruit of *F. retusa* on scaling



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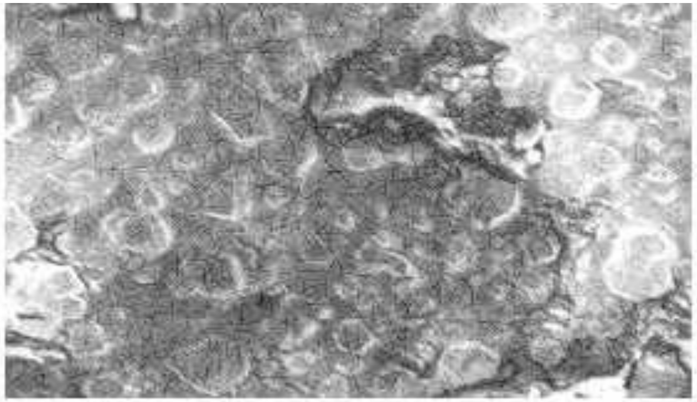

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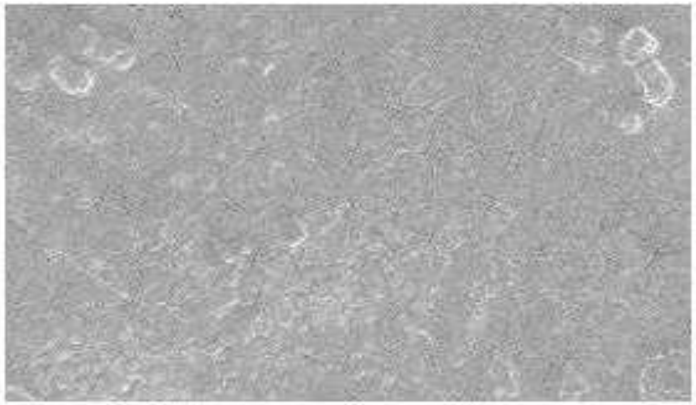
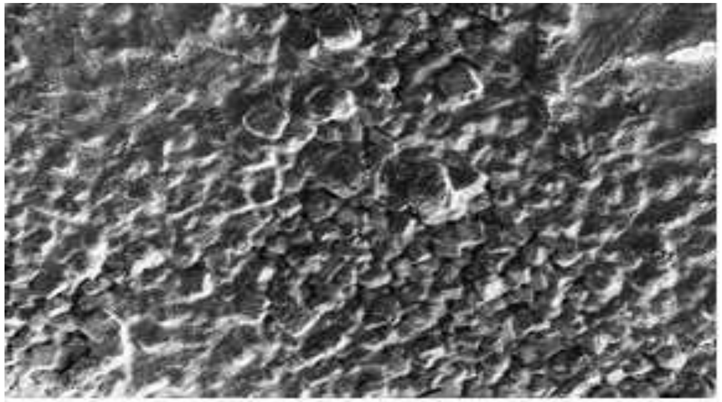
50 ppm, 50X



100 ppm, 50X

Figure 4.30. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the leaves of *F. bengalensis* on scaling



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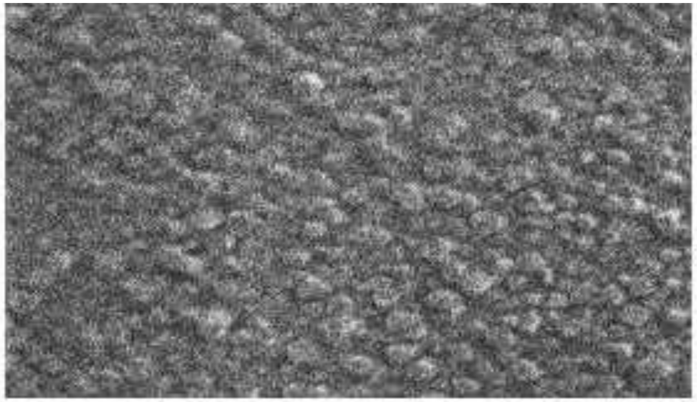

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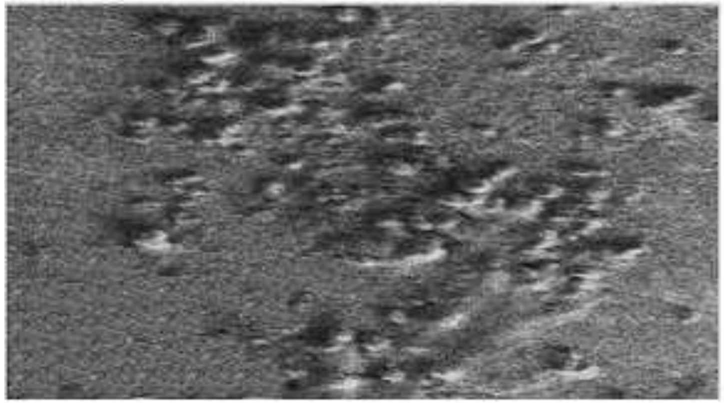

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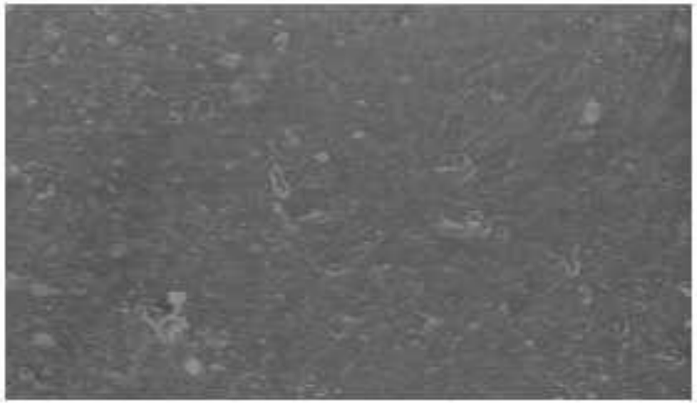
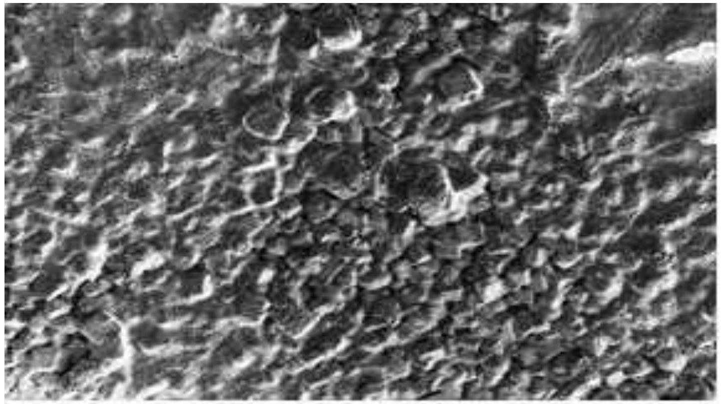


100 ppm, 50X

Figure 4.31. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the leaves of *F. infectoria* on scaling



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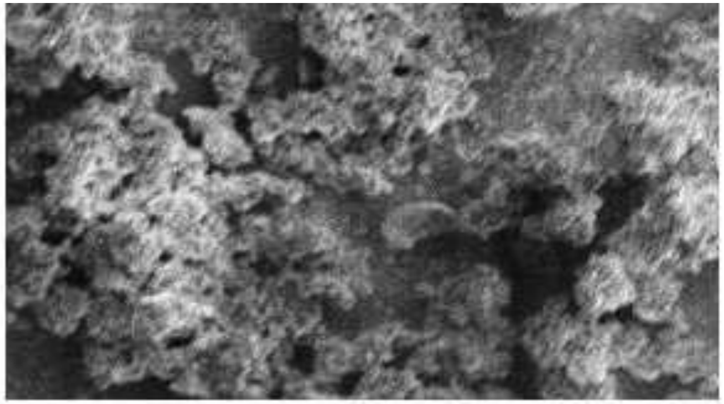

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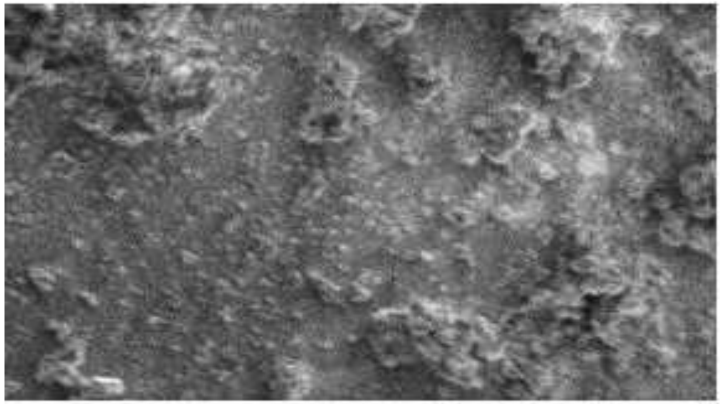

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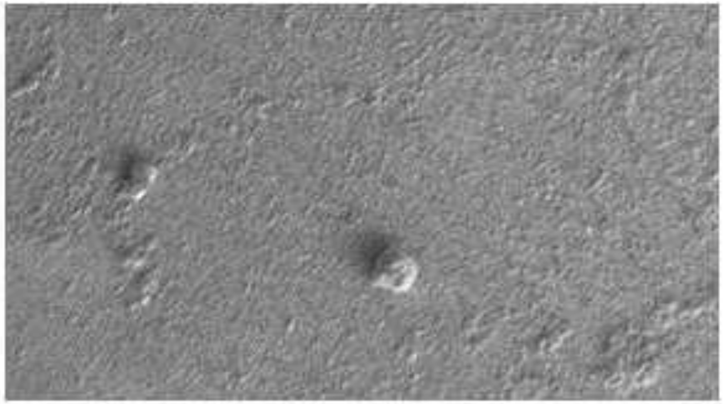

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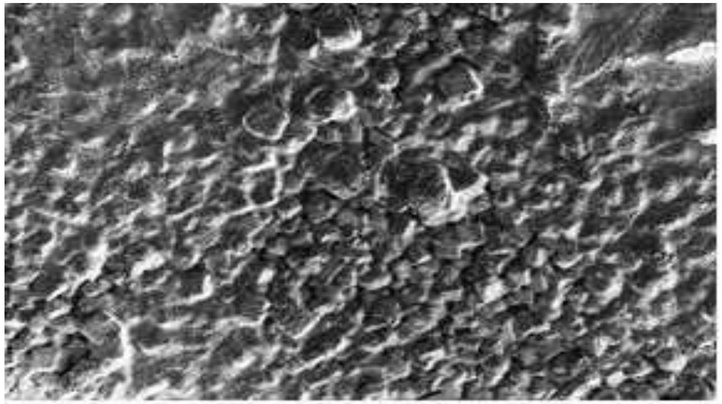
Figure 4.32. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the leaves of *F. racemosa* on scaling



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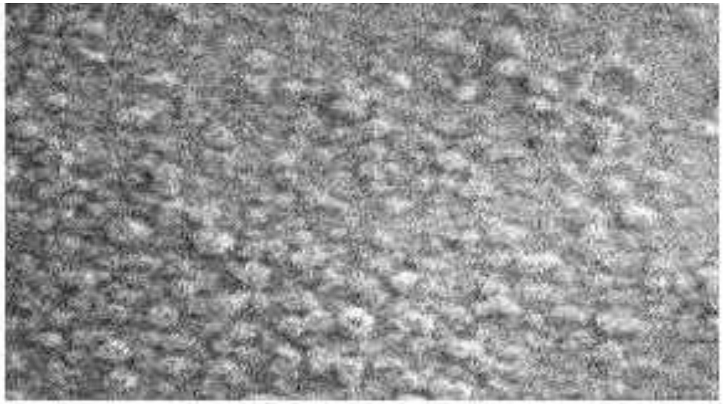

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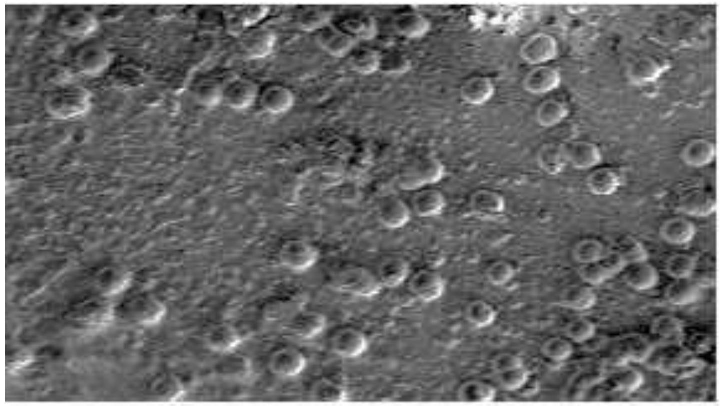

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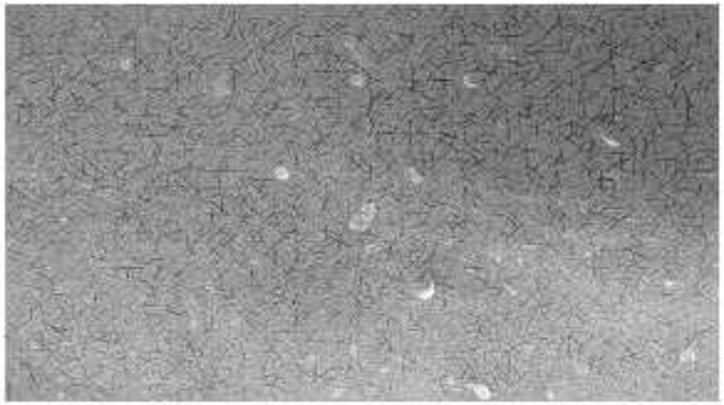


100 ppm, 50X

Figure 4.33. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the leaves of *F. religiosa* on scaling

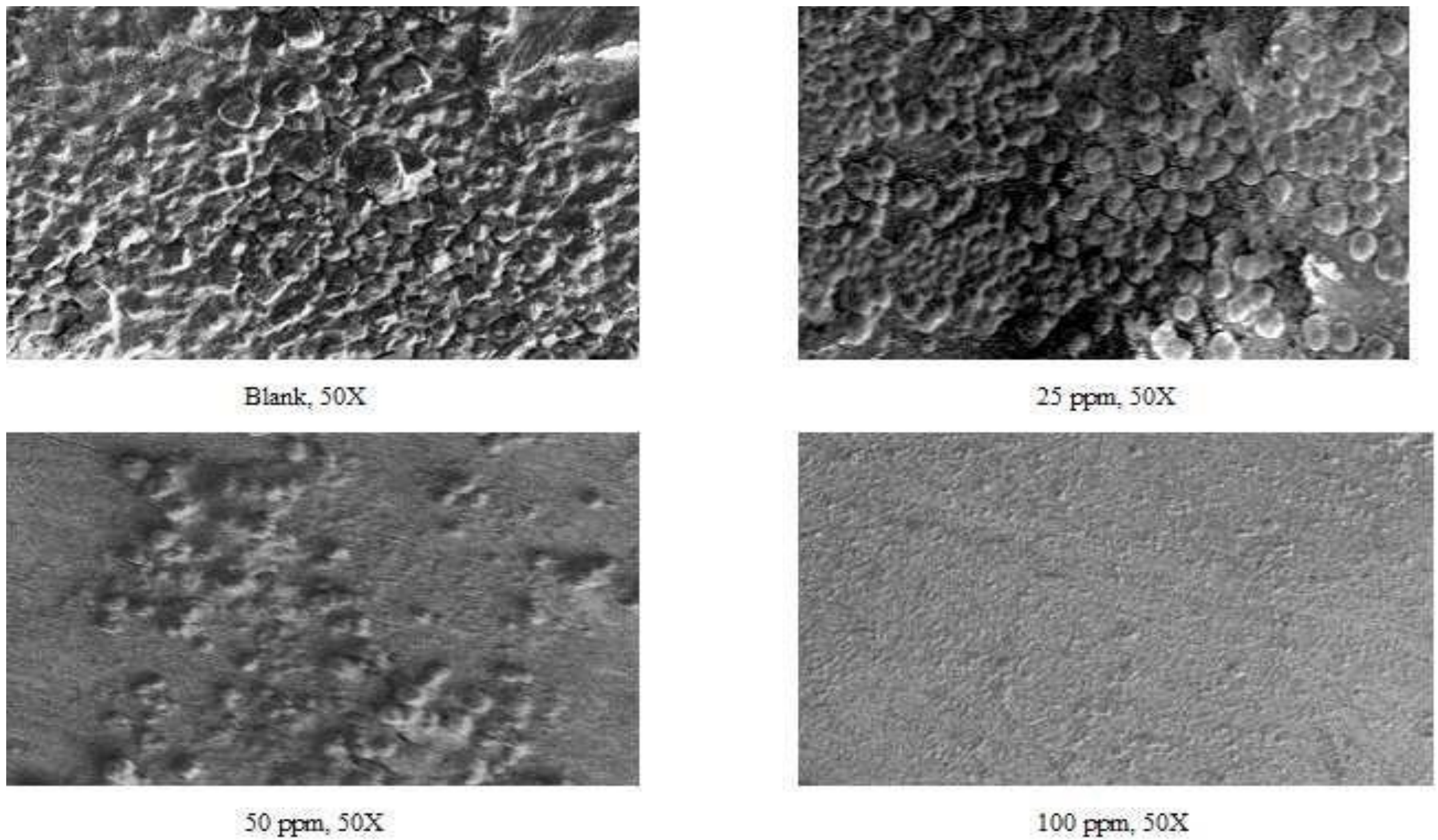
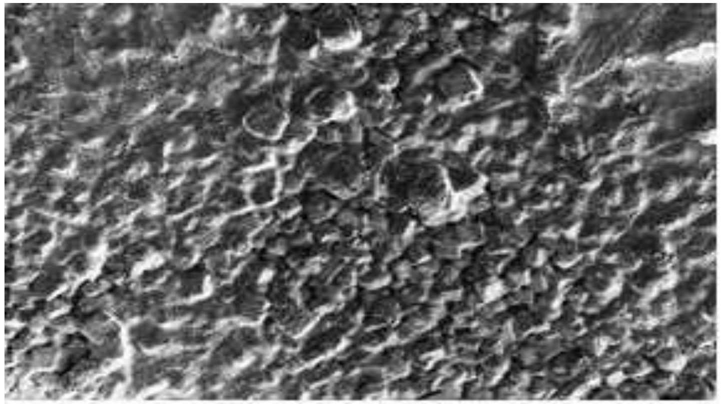


Figure 4.34. Scanning Electron Microscopic graph showing effect of different concentrations of the extracts obtained from the leaves of *F. retusa* on scaling



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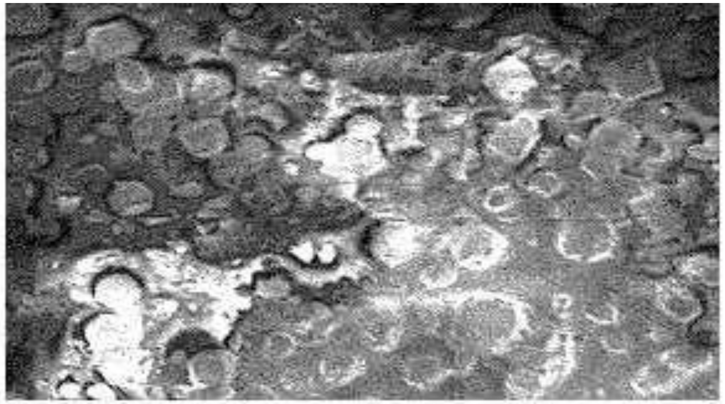

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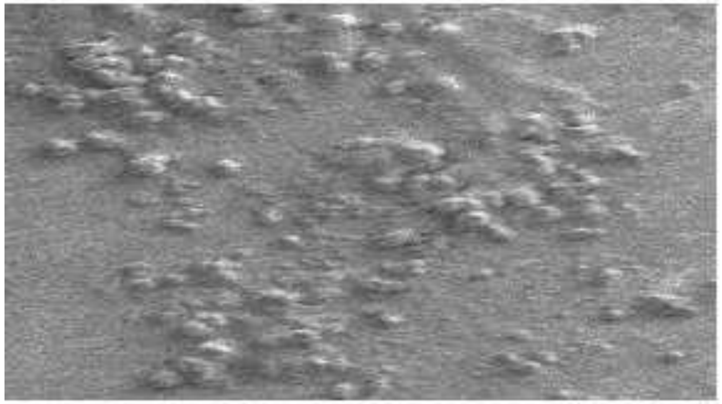

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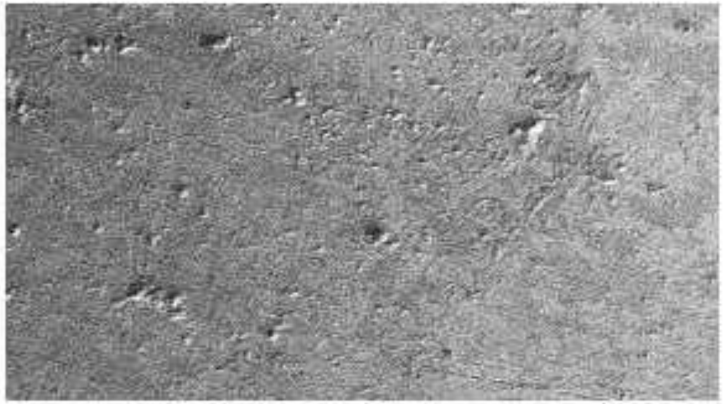

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Figure 4.35. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the bark of *F. bengalensis* on scaling

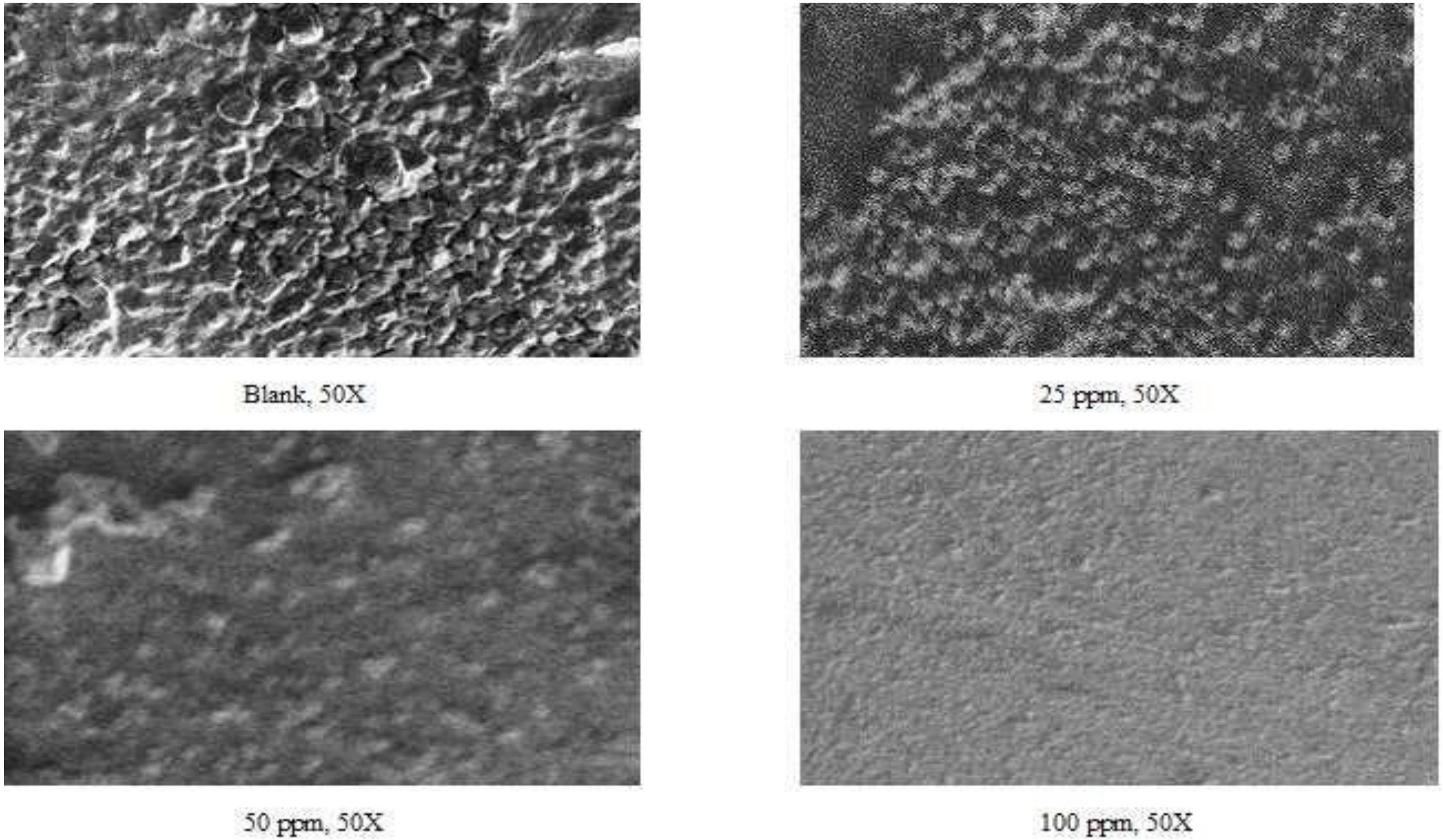
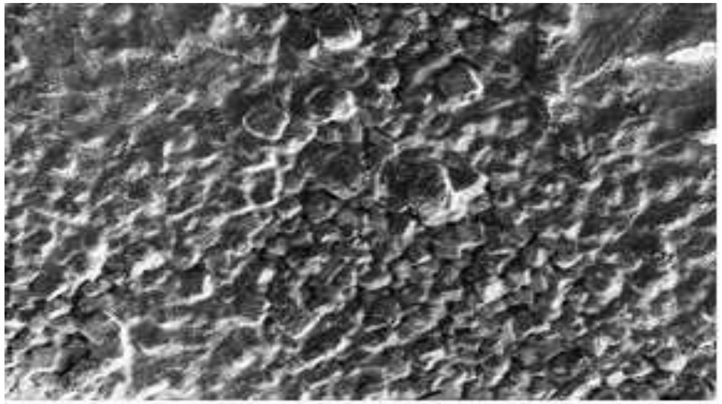


Figure 4.36. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the bark of *F. infectoria* on scaling



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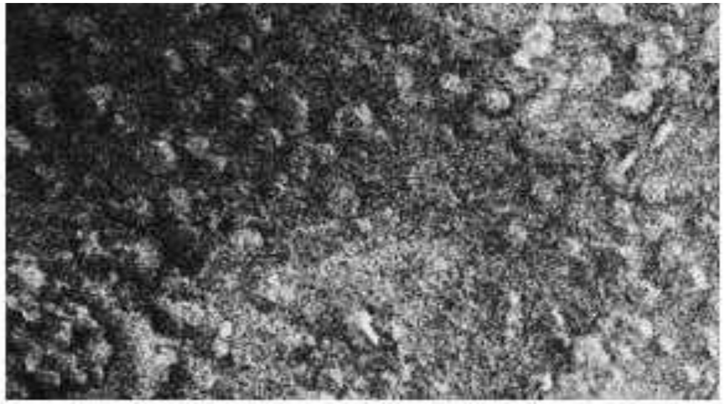

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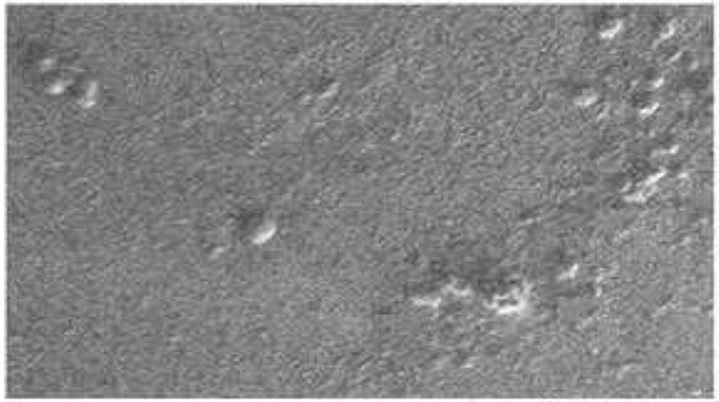
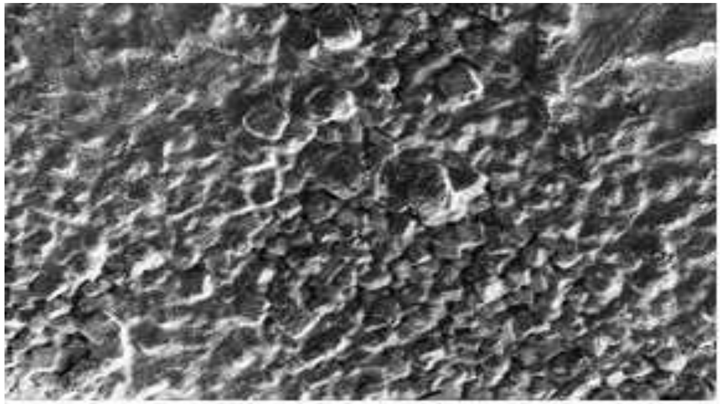


100 ppm, 50X

Figure 4.37. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the bark of *F. racemosa* on scaling



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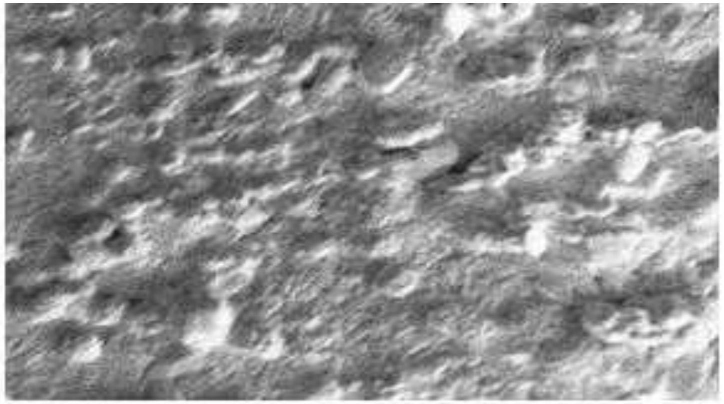

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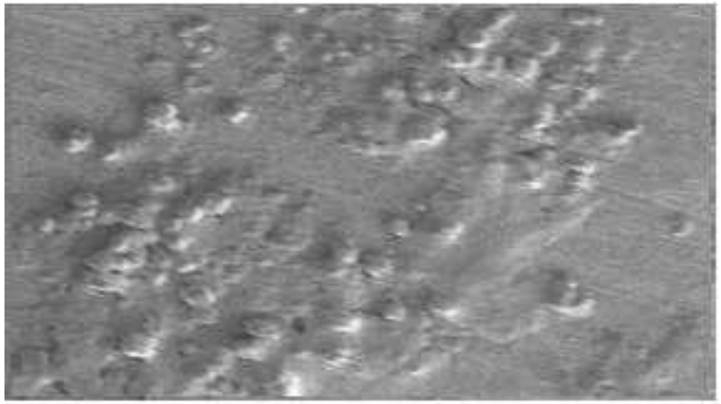

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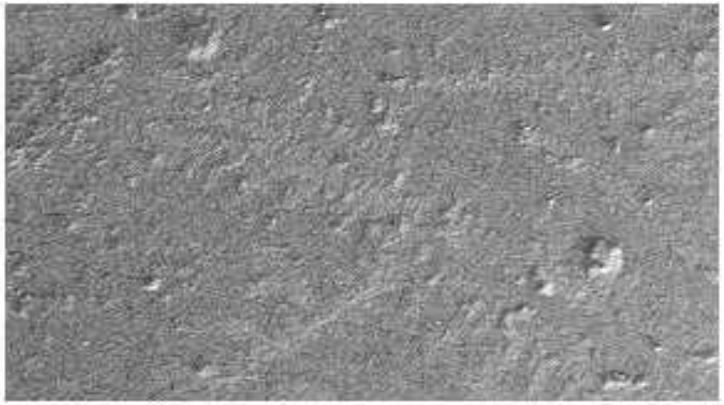

100 ppm, 50X

Figure 4.38. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the bark of *F. religiosa* on scaling

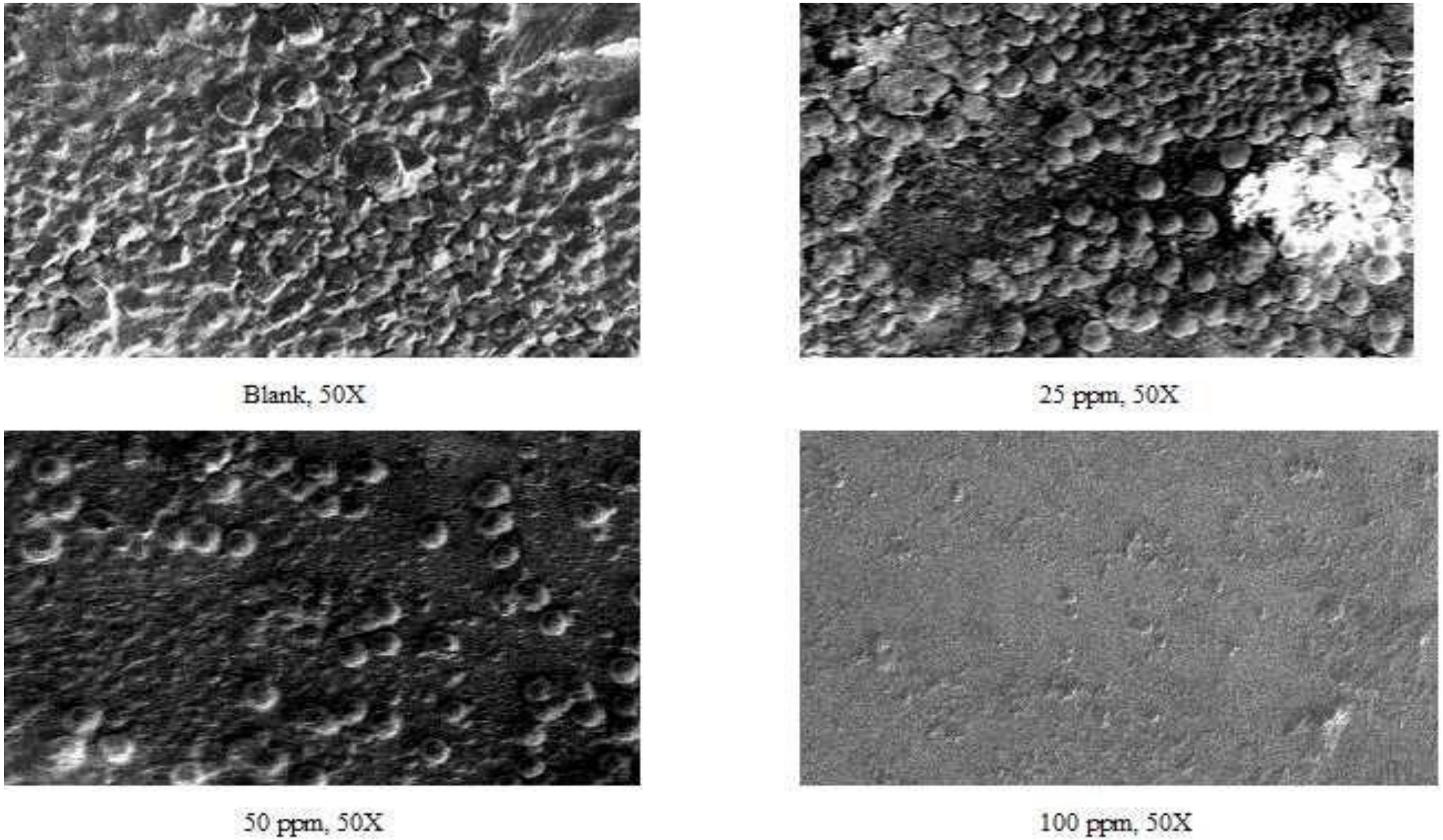

Figure 4.39. Scanning Electron Microscopic images showing effect of different concentrations of the extracts obtained from the bark of *F. retusa* on scaling

Chapter 5

Summary

Currently imposed restrictions and health concerns on the use of synthetic antioxidants as food additives prompted the need for search of natural antioxidants which are generally regarded as safe and medicinally beneficial. The revival of interest in the use of plants as source of food and medicine also encouraged the researchers to explore plant based natural antioxidants and bioactives as ingredient of functional food and nutraceuticals. The genus *Ficus*, known to be widely spread all over the world, has history of medicinal uses against different diseases in the folk medicine system of several civilizations. Due to lack of scientific realization, typically the plants of this genus are less explored and under-utilized and rarely characterized for their biochemical principals. This bolstered the need to investigate the biochemical prospects of these multipurpose plants. Hence different parts (fruits, leaves and barks) of the important plants of this genus, found abundantly in Pakistan, were selected to investigate their antioxidant, antimicrobial and antiscalant activities.

The research work presented in this dissertation was carried out in the analytical laboratory of Department of Chemistry, University of Agriculture, Faisalabad, Pakistan; Department of Food Science and Technology, University of Georgia, Athens GA USA and Centre for Advanced Ultrastructure Research (CAUR), University of Georgia, Athens GA USA. Fruits, leaves and barks of five plants (*F.bengalensis*, *F. infectoria*, *F. racemosa*, *F. religiosa* and *F. retusa*), belonging to genus *Ficus*, were harvested and assayed for assessment of their antioxidant, antimicrobial and antiscalant activities and phenolics profile. Plants samples were classified into three groups: fruits, leaves and barks. Influence of extraction process involving four solvents (100% ethanol , 100% methanol, 80% ethanol and 80% methanol) and three extraction techniques (sonication assisted extraction, magnetic stirring, orbital shaker extraction) was studied on the total phenolic contents, total flavonoids contents, DPPH radical scavenging activity, reducing power and %age inhibition of linoleic acid peroxidation of the different parts of the *Ficus* plant species selected. . The most potent (biologically active) extracts, based on the contents of total phenolics, total flavonoids and antioxidant activities, were further analyzed for identification and quantification of individual phenolic acids and flavonoids. The most potent extracts from each category were also evaluated for its antimicrobial and antiscalant activities.

Data obtained after the analysis of different samples investigated in the present study revealed that significantly higher amounts of extraction yield were obtained when combination of 80% methanol with sonication was employed for extraction. The extracts obtained by the application of 80% methanol with sonication constituted significantly ($p<0.05$) higher amounts of total phenolic contents and total flavonoid content. Similarly, significantly ($p<0.05$) higher antioxidant activities were exhibited by the extract obtained with the application of 80% methanol with sonication. In most of the cases, statistical ranking of the solvents was either 80% methanol> 80% ethanol> 100% methanol> 100% ethanol or 80%methanol> 80% ethanol> 100% methanol ≈ 100% ethanol. In most of the cases, statistical ranking for extraction techniques was sonication > magnetic stirring ≈ orbital shaking and in some instances this ranking was sonication > magnetic stirring > orbital shaking. In few cases there was no significant effect of techniques was found on different antioxidant activities studied. Among all the fruits, leaves and bark samples investigated in the present study, the extracts obtained from the fruits of *F. retusa* by employing 80% methanol with sonication constituted higher amounts of total phenolic contents and total flavonoid contents. The antioxidant activities of these extracts were also higher among all the fruits leaves and bark samples investigated in the present study.

HPLC analysis of the most potent extracts (based upon preliminary antioxidant assessment) among others revealed that all the extracts contained considerable amounts of individual phenolic acids and flavonoids. Caffeic acid, chlorogenic acid, gentisic acid and sinapic acid were principally present in almost all the fruits, leaves and barks samples of the *Ficus* species investigated. Among the fruit samples, gentisic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid were detected in significantly ($p<0.05$) higher amounts in the fruits of *F. retusa*. Significantly ($p<0.05$) higher amounts of syringic acid and ferulic acid were detected in the fruits of *F. religiosa* while significantly ($p<0.05$) higher amounts of protocatechuic acid and sinapic acid were detected in the fruits of *F. racemosa* and *F. Infectoria* respectively. Among the leaf samples, gallic acid, gentisic acid, caffeic acid and ferulic acid were detected in significantly higher amounts in the leaves of *F. bengalensis* while significantly ($p<0.05$) higher amounts of protocatechuic acid, chlorogenic acid, vanillic acid and ferulic acid were detected in the leaves of *F. racemosa*. Among the bark samples, the extract obtained from the bark of *F. religiosa* contained significantly ($p<0.05$) higher amounts of gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, caffeic acid and p-coumaric acid while significantly ($p<0.05$) higher amounts of vanillic acid, syringic acid, ferulic acid and sinapic acid were detected in the bark of *F. retusa*.

As for as the distribution of flavonoids is concerned, rutin and quercetin were found in all the fruits, leaves and bark samples of the selected species of *Ficus*. Among the fruit samples, in the fruit of *F. religiosa* significantly ($p<0.05$) higher amounts of quercetin and kaempferol were detected while significantly ($p<0.05$) higher amounts of rutin, myricetin and luteolin were detected in the fruits of *F. racemosa*, *F. retusa* and *F. Infectoria* respectively. In case of leaf samples, quercetin, luteolin and kaempferol were detected in significantly ($p<0.05$) higher amounts in the leaves of *F. Infectoria* while the extracts obtained from the leaves of *F. retusa* and *F. racemosa* constituted significantly ($p<0.05$) higher amounts of rutin and myricetin, respectively. Myricetin was not detected in bark samples of *Ficus* species tested in the present study while significantly ($p<0.05$) higher amounts of rutin, quercetin, luteolin and kaempferol were detected in the barks of *F. religiosa*.

Screening of antimicrobial activity of different parts of *Ficus* species against selected strains of microbes revealed that fruit and leaf samples have fairly good antimicrobial activity against gram positive bacteria while their activity was mild against gram negative bacteria. Interestingly, bark samples did not show any activity against gram positive or gram negative bacteria. All the samples studied for their antifungal activity were found inactive against the selected fungal strains. The strain *B. cereus* was found to be the most sensitive against all the fruit and leaf samples of *Ficus* species investigated in the present study. On the other hand, all the fruit, leaf and bark samples were noted to be inactive against *E. coli*. Meanwhile, all the fruit samples were found inactive against *E.aerogenes*. *P.aeruginosa* was found to be quite sensitive to all the fruit and leaf samples except the fruits of *F. religiosa.*

Evaluation of antiscalant activities of the fruit, leaf and bark samples revealed that all the samples tested has considerable antiscalant activity. Conductivity test revealed that extracts obtained from different parts of the tested plants appreciably shift the saturation point to the higher position. Scanning electron microscopic examination of the scale developed on the steel strip used as cathode revealed that presence of extract impeded the growth of scale crystals. Mechanical scratching of the scale formed on the steel strip exposed the non-adherent nature of scale formed in the presence of extracts.

From the results of this study it could be suggested that different parts of *Ficus* species investigated are rich in bioactive components especially in phenolic acids and flavonoids which might have been responsible for their bioactivities. Antioxidant and antimicrobial activities exhibited by different parts of *Ficus* species make them strong candidate for their use in health (as natural remedy against different diseases), in food industry (as preservative) and in water treatment plants and heat exchangers as antiscalant. However, further investigations on these species could be done to appraising the detailed phytochemicals/biochemicals profile so as to explore their specific-based applications in functional food and nutraceutical industry as well as in water treatment plants.

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